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ACUTE SYPHILITIC MENINGITIS TREATED WITH PENICILLIN[1]

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Submitted for publication October 19, 1944

The recent demonstration that penicillin is of value in the treatment of syphilis (1, 2, 3) makes it important to record promptly and in as much detail as possible the results obtained in various types of syphilitic infection. Within the first year of use of the drug, interest has naturally centered in early syphilis (1, 2) which provides the readiest yardstick of therapeutic efficacy, and is moreover of most importance to the Armed Forces and to the public health.

Aside from uncomplicated primary and secondary syphilis, investigative effort has been devoted largely to neurosyphilis. All types of neurosyphilis have been treated (3). The ultimate test of the efficacy of penicillin in neurosyphilis may well rest on its effect in the serious late forms of the disease, especially paresis. Several years may be necessary before an opinion can be formed. Early neurosyphilis, however, either asymptomatic or with the typical manifestations of acute syphilitic meningitis, provides an opportunity to obtain some information rapidly.

This paper is a preliminary report of the effect of penicillin on acute syphilitic meningitis in ten patients treated at The Johns Hopkins Hospital. In all patients treated, the drug employed was sodium penicillin. The route of administration was intramuscular for reasons to be discussed later. Dosage and duration of treatment were variable, but no other form of treatment was given with or after penicillin in any of the patients here reported.

CASE REPORTS

Case 1. (JHH 305141) A 19 year old Negress was admitted to The Johns Hopkins Hospital on October 19, 1943, complaining of severe headache and vertigo.

[1] The work in this paper was done under a contract recommended by the Committee on Medical Research of the Office of Scientific Research and Development.

These symptoms began three weeks before admission, and seven days later were followed by partial but increasing deafness, first on the right and then on both sides. The right side of her face became paralyzed four days prior to admission. There was no history of earlier manifestations of syphilis.

Vision in the left eye, the macular region of which showed a small scar, was limited to light perception and was said to have been bad for years. The fundi were otherwise normal. Bilateral nerve deafness, a right facial paralysis of the peripheral type, a macular rash, and a few darkfield positive genital papules, which the patient had not noticed, were present. There was 50 per cent hearing loss on the right side and 45 per cent on the left by audiometer test. Physical examination was otherwise normal.

The Eagle flocculation titer of the blood was 4 units. The spinal fluid, which was clear and colorless, contained 53 mononuclear cells per cubic millimeter and 25 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.4 cc. but not with smaller amounts, and the mastic test was negative. The erythrocytes sickled, and a biologic test for pregnancy, suggested by the fact that her last menstrual period had been early in September, was positive.

Treatment with 20,000 Oxford units of sodium penicillin intramuscularly every three hours was started on October 22 and continued for 62 injections, a total of 1,240,000 units in seven and one half days. There was no Herxheimer reaction. The mucocutaneous lesions healed promptly. Improvement in hearing and in facial strength, which was first apparent on the fourth day of therapy, continued, so that by the eleventh day after the start of treatment there was no evidence of facial weakness. By the thirteenth day hearing had improved on the left to only 15 per cent loss, although there was still 55 per cent hearing loss on the right.

On November 5, fifteen days after the start of treatment, the spinal fluid contained 29 mononuclear cells per cubic millimeter and 37 mgm. of protein per 100 cc. The Wassermann reaction was now negative with 1.0 cc. and the mastic test gave a flat reading. After discharge the patient was followed in the clinic where her clinical course was uneventful. On January 5, 1944, the Eagle blood flocculation test was negative. The spinal fluid contained no cells and 17 mgm. of protein per 100 cc. The mastic test was negative in all dilutions and the Wassermann reaction was negative with 1.0 cc. Hearing continued to improve; on January 14, there was 12 per cent hearing loss on the right and 3 per cent on the left, and on February 8, six per cent loss on the right and 3 per cent on the left. Frequent blood Eagle flocculation tests gave negative or doubtful results except on one occasion, April 13, when a titer of one unit was reported.

On April 22, because of severe pre-eclampsia, she was admitted to the obstetrical service where she was found to have a marginal placenta praevia. The following day she was delivered by version and extraction. The child showed no evidence of syphilis, darkfield examination of scrapings from the umbilical vein showed no spirochetes, and serologic tests on cord blood were negative. The child has been carefully followed for 124 days and so far has shown no clinical, serologic, or roentgenologic evidence of congenital syphilis. On April 27, the patient's spinal fluid was normal.

TABLE I

The Course under Penillicin Therapy of Case 1

DATE	DAY AFTER PENICILLIN TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
10/20/43			4	53 cells 25 mgm. % protein WaR pos. with 0.4 cc. Mastic 0000000000	Headache 3 weeks Hearing—Rt. = 50% loss Lt. = 45% loss Rt. VII nerve palsy DF+ genital lesion Early pregnancy Sickle cell trait
10/22/43	0-8	Penicillin 1,240,000 units			VII nerve normal 4th day; headache, etc. gone.
11/ 3/43	13				Hearing—Rt. = 55% loss Lt. = 15% loss
11/ 5/43	15		D*	29 cells 37 mgm. % protein WaR neg. with 1.0 cc. Mastic 0000000000	
11/22/4	31				Improved
1/ 5/44	75		0	Normal	
1/14/44	84				Hearing—Rt. = 12% loss Lt. = 3% loss
1/20/44	90		0		
2/ 3/44	104		D		
2/ 7/44	109				Hearing—Rt. = 6% loss Lt. = 3% loss
2/17/44	116		0		
3/16/44	144		0		
4/13/44	172		1		Hearing—Rt. = 3% loss Lt. = no loss
4/27/44	186		D	Normal	Delivery, April 23, normal child
5/10/44	197		D		Sickle cell anemia crisis
6/ 8/44	226		D		Slight headache
6/15/44	233		0		
6/29/44	247		D		Asymptomatic
7/13/44	261		D		
8/31/44	310		0		Asymptomatic

*D = doubtful reaction.

On May 10 she returned to the hospital in a severe hemolytic crisis, a manifestation of sickle cell anemia. She recovered rapidly from this and has since been asymptomatic although Eagle flocculation tests of the blood have continued to be frequently doubtful. The course is summarized in Table I.

Case 2. (JHH 308125) A 21 year old negress was admitted to The Johns Hopkins Hospital on November 26, 1943. A year previously she had developed intermittent, diffuse headaches which 10 months later had increased in frequency and duration and three days prior to admission had become constant and refractory to aspirin. She vomited once on the day of admission. Four months prior to that, because of a rash on the volar surfaces of her forearms, she had consulted her private physician who, finding her blood serologic test to be positive, had given her six intravenous injections, the last one three weeks before admission. This treatment caused the disappearance of the rash (presumably secondary syphilis), but the headache did not improve. There was no history of earlier manifestations of syphilis.

Essential findings of the admission physical examination were a very stiff neck, three diopters of papilledema on the right with a few small retinal hemorrhages and slight papilledema on the left. The other cranial nerves were normal. The skin and mucous membranes were normal, and there were a few small superficial lymph-nodes. Visual acuity was 20/30 right and 20/30 left. Visual fields were normal except for enlargement of the right blind spot.

The Eagle blood flocculation titer was 16 units. Roentgenograms of the skull, sinuses, and chest were normal. Because a brain tumor might have been present, it was thought advisable to precede lumbar puncture by ventriculography. The ventricles were normal in size and shape, so that a lumbar puncture subsequently was done. The spinal fluid, which had a ground-glass appearance and was under an initial pressure of 290 mm. of water, contained 1280 cells per cubic millimeter, of which 87 per cent were mononuclear and 12 per cent polymorphonuclear, and 100 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.05 cc. but negative with smaller amounts, and the mastic curve was 5555553100.

Treatment was started on December 14 with 10,000 Oxford units of sodium penicillin intramuscularly every three hours for sixty-four injections, 640,000 units in seven and one half days. There was no Herxheimer reaction. Her temperature had varied between normal and 102° previously, but from the second day of therapy on she was afebrile.

By December 16, two days after treatment was started, the headache ceased, and the following day diminution in the papilledema was apparent. On December 26 the spinal fluid, which was clear and colorless, contained 80 mononuclear cells per cubic millimeter and 37 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.1 cc. but not with 0.5 cc., and the mastic curve was 5555432100.

After discharge she remained well. By January 17, 1944, there was no papilledema. On February 3, the spinal fluid contained 37 mgm. of protein per 100 cc., but no cells were seen. The Wassermann reaction was positive with 0.2 cc. but

TABLE II

The Course under Penicillin Therapy of Case 2

DATE	DAY AFTER PENICILLIN TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
Sept. '43		6x Arsenic	+		Secondary syphilis (?)
11/26/43			4		Severe headaches Papilledema Negative ventriculogram
12/14/43	0–8	Penicillin 640,000 units	16	1280 cells 100 mgm. % protein WaR. pos. with .05 cc. Mastic 5555553100	Papilledema Headache
12/26/43	12		32	80 cells 37 mgm. % protein WaR. pos. with 0.1 cc. Mastic 5555432100	Asymptomatic Much less papilledema
1/ 4/44	20		16		Improvement continues
1/17/44	34		12		
2/ 3/44	48		4	0 cells 37 mgm. % protein WaR. pos. with 0.2 cc. Mastic 5543100000	Optic discs normal
2/24/44	67		4		
3/ 9/44	81		4	0 cells 37 mgm. % protein WaR. pos. with 0.4 cc. Mastic 5555421000	

TABLE II—*Continued*

DATE	DAY AFTER PENICILLIN TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
3/23/44	95		2		
4/20/44	123		2		
7/ 6/44	200		1	12 cells 22 mgm. % protein WaR. pos. with 0.6 cc. Mastic 2211000000	Asymptomatic
9/ 7/44	263		D		Asymptomatic

not with 0.1 cc., and the mastic curve was 5543100000. On March 9 there were 37 mgm. of protein per 100 cc. of spinal fluid, but no cells were found. The Wassermann reaction was positive with 0.4 cc. but not with 0.2 cc., and the mastic curve was 5555421000. The Eagle blood flocculation titer diminished and on April 20 was 2 units. The patient was last seen on September 7, 1944, and was asymptomatic, the blood Eagle flocculation test being doubtful. The spinal fluid abnormalities continue to regress. The findings are listed in Table II.

Case 3. (JHH 315662) A 27 year old negro male was admitted to The Johns Hopkins Hospital on March 10, 1944, complaining of having had several convulsions. In August, 1943, because of a penile sore (presumably primary syphilis), he had been given elsewhere some antisyphilitic treatment (details not exactly known but consisting of about 20–25 hip and arm injections), which continued irregularly until December. The lesion healed promptly, but late in February, 1944, he became somewhat drowsy and developed headaches. He had one generalized convulsion on March 4 and three subsequent ones which resulted in his admission on March 10.

The patient appeared acutely and seriously ill. The neck was moderately stiff and the sensorium quite clouded. No cranial nerve abnormalities could be made out, and no other physical findings of note were present.

The Eagle blood flocculation test was negative. The spinal fluid, which was clear and colorless, contained 102 cells per cubic millimeter and 105 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.05 cc. but not with smaller amounts, and the mastic curve was 5555543100.

Treatment was started on March 11 with 10,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 doses, 600,000 units in seven and one half days. A rise in temperature to 100.6° nineteen hours after the start of treatment was thought to be a Herxheimer reaction. During treatment the head-

ache and drowsiness cleared strikingly, and no further convulsions occurred. On March 19 the spinal fluid contained 42 cells per cubic millimeter and 19 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.4 cc. but not with smaller amounts, and the mastic curve was 3210000000.

TABLE III

The Course under Penicillin Therapy of Case 3

DATE	DAY AFTER PENICILLIN THERAPY	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
Aug. '43		20–25 Bismuth & Arsenic	+ (no titer)		Chancre
3/10/44	0–8	Penicillin 600,000 units	0	102 cells 105 mgm. % protein WaR. pos. with .05 cc. Mastic 5555543100	Convulsions Headache Drowsy
3/19/44	9		D	42 cells 19 mgm. % protein WaR. pos. with 0.4 cc. Mastic 3210000000	Greatly improved
3/30/44	20		0		Asymptomatic
4/12/44	33		D	Normal	Asymptomatic
4/20/44	41		0		Asymptomatic
5/18/44	69		0		Asymptomatic
6/15/44	97		0		Asymptomatic
7/13/44	125		0		Asymptomatic
8/24/44	167		0		Asymptomatic

After discharge the patient remained well except for several spells of weakness and nervousness. On April 12 the spinal fluid, which contained 4 cells per cubic millimeter, gave a negative Wassermann reaction with 1.0 cc. and a negative mastic test. He was well when examined on August 24, 1944. Table III illustrates the course.

Case 4. (JHH 312211) An 18 year old negro male was admitted on January 22, 1944, in a stupor following repeated convulsions. On November 2, 1943, he

had been seen in the Syphilis Clinic of The Johns Hopkins Hospital with a papular rash, enlargement of the superficial lymphnodes, and a darkfield positive penile lesion. Antisyphilitic treatment was begun and five injections of 0.06 gm. of marpharsen and two injections of 0.2 gm. of bismuth were given within nine days before the patient stopped treatment. An effort made to have him treated in a city clinic was, due to his uncooperativeness, a failure. The lesions healed promptly, and he was asymptomatic until January 22, 1944. when, after a mild headache, he had a series of generalized convulsions which resulted in his hospital admission.

At that time he was stuporous, but neither then nor the next day when he had recovered consciousness could any neurologic or other abnormalities be found.

The Eagle blood flocculation titer was 2 units. The spinal fluid contained 70 lymphocytes per cubic millimeter and 25 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.4 cc. but negative with smaller amounts. The mastic curve was 1100000000.

Treatment was started on the day of admission with 10,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 injections, 600,000 units in seven and one half days. There was no Herxheimer reaction, nor did subsequent convulsions occur. On January 30 the spinal fluid contained 15 cells per cubic millimeter and 25 mgm. of protein per 100 cc. The Wassermann reaction was negative with 1.0 cc., and the mastic test was negative.

The patient has remained well. The Eagle test was negative on March 16 and subsequently. The spinal fluid on July 6 was normal. Table IV summarizes the findings.

Case 5. (JHH 322004) A 38 year old negress was admitted to the neurosurgical service on May 21, 1944, complaining of double vision of three weeks' duration. This and a mild headache, which had since been intermittently present, had developed on May 1. On May 13 she was seen by a physician who found that her right sixth nerve was paralyzed. On May 17 she had severe attack of rotary vertigo followed by nausea and repeated vomiting. Tinnitus and partial deafness developed on the right, associated with dizziness and staggering. The next day the right side of her face felt tight. She was admitted to another hospital and found to have right sixth and seventh nerve paralyses and lateral nystagmus on looking to the left. She was discharged after arrangements had been made for her to come to The Johns Hopkins Hospital where she was admitted to the neurosurgical service on May 21.

Essential findings on physical examination were complete paralysis of the right sixth and seventh nerves, complete loss of hearing on the right by audiometer test, normal hearing on the left, nystagmus to the left on lateral gaze, staggering to the right, large pupils reacting neither to light nor on accommodation, normal visual fields, and protrusion of the tongue slightly to the left, although it could be moved to the right.

Following examination of the spinal fluid a diagnosis of syphilitic meningitis was made and the patient transferred to the medical service on May 25.

The Eagle blood flocculation titer was 2 units. The spinal fluid, which was clear

and colorless, contained 70 lymphocytes per cubic millimeter and 118 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. but negative with smaller amounts, and the mastic curve was 5554310000.

TABLE IV

The Course under Penicillin Therapy of Case 4

DATE	DAY AFTER PENICILLIN TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
11/ 2/43		Mapharsen .06 × 5 Bismuth ×2	32		Secondary syphilis
1/21/44				34 cells 45 mgm. % protein WaR. pos. with 0.4 cc. Mastic 3210000000	Convulsions Delirium Headache
1/22/44	0	Penicillin 600,000 units	2	70 cells 25 mgm. % protein WaR. pos. with 0.4 cc. 1100000000	
1/23/44	1				Mentally clear Much improved
1/30/44	8		4	15 cells 25 mgm. % protein WaR. neg. with 1.0 cc. Mastic 0000000000	Asymptomatic
2/10/44	19		D		Asymptomatic
3/16/44	54		0	Normal	Asymptomatic
5/ 9/44	78		0	Normal	Asymptomatic
7/ 6/44	106		0	Normal	Asymptomatic
9/14/44	176		0		Asymptomatic

Treatment was started on May 26 with 20,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 injections, 1,200,000 units in seven and one half days. Her temperature rose to 100.2° twenty-four hours after the start of therapy. This was the highest temperature recorded while she was in the hos-

TABLE V

The Course under Penicillin Therapy of Case 5

DATE	DAY AFTER PENICILLIN TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
5/25/44			2	70 cells 118 mgm % protein WaR. pos. with 0.2 cc. Mastic 5554310000	Paralysis, rt. VI, VII, VIII nerves
5/26/44	0–8	1,200,000 units penicillin			
6/ 3/44	9		1	80 cells 86 mgm. % protein WaR. pos. with 0.2 cc. Mastic 5555431000	Symptomatic improvement
6/ 8/44	14		D		Improved
6/15/44	21		1		VII nerve unimproved
6/29/44	35		D	8 cells 58 mgm. % protein WaR. pos. with 0.4 cc. 2322100000	VI nerve greatly improved VII unimproved
7/27/44	63		D		VI nerve, only slight weakness VII nerve improved
8/31/44	98		0	8 cells 51 mgm. % protein WaR. pos. with 0.6 cc. Mastic 5554300000	Improvement continues
9/ 6/44	104				Audiometer: no improvement

pital and was thought to represent a Herxheimer reaction. During treatment there was a slight improvement in the sixth nerve paralysis, she was able to walk without staggering, the headache ceased, and the tinnitus diminished. On June 3 the spinal fluid contained 80 cells and 86 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. but not with smaller amounts, and the mastic curve was 5555431000. On June 14 she could hear but could not understand a phonographic test record with the right ear, but there was only slight improvement in function of the sixth and seventh nerves. On June 29, 1944, the spinal fluid contained 8 lymphocytes, 58 mgm. of protein per 100 cc., a positive Wassermann reaction with 0.4 cc., and a mastic curve of 2322100000. Ninety-eight days after treatment (August 31, 1944), the Eagle flocculation titer was negative and great improvement had taken place in the function of the sixth and seventh cranial nerves on the right. An audiometer test on September 6, 1944, showed no improvement in the hearing on the right.

Case 6. (JHH 161626) A 30 year old negro male was admitted to The Johns Hopkins Hospital on May 15, 1944, complaining of moderate headache of a month's duration. In November 1942, as a family contact, a routine blood serologic test was negative. He had had an untreated penile sore in March, 1944, but no other symptoms. Examination showed a very slight left facial weakness of the peripheral type, a few darkfield-positive papules on the face and abdomen, a darkfield positive scar on the prepuce, and moderately enlarged inguinal lymphnodes. An audiometer test was normal.

The spinal fluid, ground-glass in appearance, contained 1450 lymphocytes per cubic millimeter and 158 mgm. of protein per 100 cc. The Wassermann test was anticomplementary with 0.03 to 1.0 cc. and gave a mastic curve of 5555554400.

On May 16 treatment was started with 20,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 doses, or 1,200,000 units in seven and one half days. There was no Herxheimer reaction and the papules, which became darkfield negative in 13 hours, healed promptly. During treatment the headaches ceased, and the slight facial paralysis cleared. On May 24 the spinal fluid was clear and colorless and contained 177 cells per cubic millimeter and 62 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.1 cc. but negative with 0.05 cc., and the mastic curve was 5555420000.

After discharge the patient remained asymptomatic and when seen on June 1 was well. The Eagle titer, which before treatment on May 15 had been 16 units and had risen two days later to 256 units and three days after that to 512 units, had fallen to 32 units. One hundred and fourteen days after treatment (September 7, 1944) the Eagle flocculation test had become negative. On September 15 the spinal fluid contained 7 cells and 26 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.8 cc.; the mastic curve was 4431000000. Table VI summarizes the findings.

Case 7. (JHH 137279) A 21 year old negress was admitted to The Johns Hopkins Hospital on March 7, 1944, complaining of deafness of 3 weeks' duration.

In September, 1941, she was seen in The Johns Hopkins Hospital Out-Patient Department with pelvic inflammatory disease, and a routine serologic test for syphilis

TABLE VI

The Course under Penicillin Therapy of Case 6

DATE	DAY AFTER PENICILLIN TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
11/18/42			0		Family contact
March '44					Untreated penile lesion
5/15/44	0	Penicillin 1,200,000	16	1450 cells 158 mg. % protein WaR. pos. with .02 cc. Mastic 5555554400	Facial weakness, lt. Headaches and dizziness—3 wks. DF + penile lesion Hearing normal
5/25/44	8		512	177 cells 62 mgm. % protein WaR. pos. with .10 cc. Mastic 5555420000	Asymptomatic Facial weakness gone
6/ 1/44	16		32		Asymptomatic
7/ 6/44	51		4	55 cells 40 mgm. % protein WaR. pos. with 0.6 cc. Mastic 4321000000	Asymptomatic
9/ 7/44	114		0		Asymptomatic
9/15/44	121			7 cells 26 mgm. % protein WaR. pos. with 0.8 cc. Mastic 4431000000	Asymptomatic

was negative. Her serologic test had also been negative in May, 1943, but the following January she had received elsewhere three intravenous injections of

mapharsen in two weeks for an illness characterized by a sore throat, rash, and genital sore. The lesions healed promptly, and she remained well until the middle of February when a transitory stiff neck, persistent headache, bilateral partial deafness and tinnitus, and a right facial paralysis developed. She came to the hospital and was admitted for treatment.

The only abnormalities on examination were a right facial paralysis of the peripheral type, bilateral nerve deafness with 25 per cent hearing loss in each ear by audiometer test, and slight enlargement of the superficial lymphnodes.

The result of the Eagle blood flocculation test was doubtful. The spinal fluid was clear and colorless and contained 57 cells per cubic millimeter and 37 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. but negative with 0.1 cc., and the mastic curve was 5543110000.

Treatment began on March 9 with 10,000 Oxford units of sodium penicillin every three hours for 60 injections, 600,000 units in seven and one half days. There was no Herxheimer reaction. Hearing was subjectively better by the third day of therapy and by March 20 there was only 12 per cent hearing loss on each side. The facial paralysis did not improve during treatment but by March 23 was less and by April 6 was completely gone. On March 17 the spinal fluid contained 20 cells and 33 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.6 cc. but not with smaller amounts, and the mastic curve was 1110000000. All Eagle tests of the blood subsequent to the first one gave negative results. When seen on July 20, 1944, the patient was quite well. The spinal fluid on June 15, 1944, contained no cells and 22 mgm. of protein per 100 cc. The Wassermann reaction was negative with 1.0 cc., and the mastic curve was 0000000000. The course is given in Table VII.

Case 8. (JHH 312861) A 21 year old white man was admitted to The Johns Hopkins Hospital on January 31, 1944, complaining of headache of three weeks' duration. Early in 1942 he had had a penile sore followed in June, 1942, by a generalized rash and sore throat. In September, 1942, his blood serologic test was found to be positive. Treatment was started and given regularly so that from this time until December, 1943, he received seventeen injections of 0.6 gm. neoarsphenamine, nineteen injections of 0.06 gm. mapharsen, and twenty injections of bismuth. Nevertheless, six months after the start of therapy and in spite of its active continuation, ulcerative lesions appeared on his lower legs and persisted for five weeks. After these healed he remained well until three weeks before admission when he developed an intermittent, severe left-sided headache, became increasingly irritable, and began to have occasional spells of dizziness. These symptoms resulted in his admission on January 31, 1944.

At that time there were several pigmented almost circular scars up to three centimeters in greatest diameter on each lower leg. The only other abnormality found on physical examination was a small penile scar on the coronal sulcus. Visual fields, audiometric tests, and vestibular tests were normal.

The Eagle blood flocculation titer was 12 units. The spinal fluid, which was clear and colorless, contained 225 leukocytes per cubic millimeter, of which 220 were lymphocytes, and 50 mgm. of protein per 100 cc. The Wassermann reaction

TABLE VII

The Course under Penicillin Therapy of Case 7

DATE	DAY AFTER PENICILLIN TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
Sept. '41			0		P.I.D.
May '43			0		
Jan. '44		Mapharsen .04 × 3	+ (no titer)		Early syphilis
3/ 7/44			D	57 cells 37 mgm. % protein WaR. pos. with 0.2 cc. Mastic 5543110000	Headache; stiff neck Palsy right VII nerve
3/ 8/44					Hearing loss: rt. 25%; lt. 25%
3/ 9/44	0–8	Penicillin 600,000 units			
3/17/44	8		0	20 cells 33 mgm. % protein WaR. pos. with 0.6 cc. Mastic 1110000000	Marked improvement
3/20/44	11				Hearing loss: rt. 12%; lt. 12%
3/23/44	14		0		Right VII improved
4/ 6/44	28		0		Right VII paralysis gone
5/ 4/44	56		0		Asymptomatic
6/ 8/44	91		0		Asymptomatic
6/15/44	98			Normal	Asymptomatic
7/20/44	133		0		Asymptomatic

was positive with 0.05 cc. but not with smaller amounts, and the mastic curve was 5554321000.

Treatment was started on February 2, 1944, with 50,000 Oxford units of sodium penicillin intramuscularly every three hours for 80 doses, 4,000,000 units in ten days. A transitory rise in temperature from normal to 102.4°F. twenty hours after the start of therapy was the only manifestation of a Herxheimer reaction. In several days the patient became completely asymptomatic. On February 12 the spinal fluid contained 130 lymphocytes per cubic millimeter and 50 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.1 cc. but not with 0.05 cc., and the mastic curve was 5544431000.

After discharge he continued to be asymptomatic. On May 4 the Eagle titer was 3 units, and the spinal fluid contained 22 cells per cubic millimeter and 37 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. but not with smaller amounts, and the mastic curve was 4321000000. One hundred and thirty-nine days after treatment (July 20, 1944) the serologic test for syphilis was 1 unit, the spinal fluid contained 8 cells and 40 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.4 cc., and the mastric curve was 4321-000000. See Table VIII.

Case 9. (JHH 313431) A 30 year old negress was admitted to The Johns Hopkins Hospital on February 7, 1944, complaining of a moderately severe sore throat which had begun one month previously and had been followed in a week by hoarseness and a rash. There was no history of earlier manifestations of syphilis.

Although she was hoarse, she did not appear very ill or uncomfortable. Examination showed a generalized, darkfield positive, papular rash, injection and edema of the pharynx and tonsils which were covered with exudate, and slightly enlarged cervical lymphnodes.

The Eagle blood flocculation was 256 units and the spinal fluid was normal.

Treatment with 1,000 Oxford units of sodium penicillin intramuscularly every three hours was begun on February 9 and continued for 60 injections, 60,000 units in seven and one half days. There was no Herxheimer reaction, and the lesions which became darkfield negative in eleven hours healed completely in two weeks.

It was difficult to get the patient to return to the clinic after discharge, but when seen on March 16 she was asymptomatic, there were no lesions, and the Eagle titer was 64 units. Early in April, after transitory diplopia and unsteadiness, the left side of her face became paralysed, she became partly deaf, and a week later genital lesions appeared.

On May 4, when she was readmitted, a left facial paralysis of the peripheral type and numerous darkfield positive vulvar condylomata lata were present. An audiometer test showed 30 per cent loss of hearing on the right and 15 per cent loss on the left.

The Eagle blood flocculation titer was 32 units. The spinal fluid, which was clear and colorless, contained 48 cells per cubic millimeter and 40 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. of spinal fluid but not with smaller amounts, and the mastic curve was 2431000000.

TABLE VIII

The Course under Penicillin Therapy of Case 8

DATE	DAY AFTER PENICILLIN TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
June '42 to Jan. '44		36 × Arsenic 20 × Bismuth	+ (no titer)		Early syphilis Eruption legs 6 mos. after treatment started.
1/25/44			6	350 cells 75 mgm. % protein WaR. pos. with .05 cc. Mastic 5554321000	Headache; dizziness nervousness
1/31/44			12	225 cells 50 mgm. % protein WaR. pos. with 0.5 cc. Mastic 5554321000	Headache; dizziness nervousness
2/ 2/44	0–8	Penicillin 4,000,000 units			
2/12/44	10		12	130 cells 50 mgm. % protein WaR. pos. with 0.1 cc. Mastic 5544431000	Rapid loss of all symptoms
5/ 4/44	92		3	22 cells 37 mgm. % protein WaR. pos. with 0.2 cc. Mastic 4321000000	Asymptomatic
7/20/44	139		1	8 cells 40 mgm. % protein WaR. pos. with 0.4 cc. Mastic 4321000000	Asymptomatic

Treatment was begun on May 6 with 20,000 Oxford units of sodium penicillin intramuscularly every three hours for 60 injections, 1,200,000 units in seven and one half days. There was no Herxheimer reaction. The condylomata, which became darkfield negative in 12 hours, healed promptly, but at the end of treatment there was no change in the facial paralysis. On May 14 the spinal fluid contained 14 cells per cubic millimeter and 33 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.4 cc. but negative with 0.2 cc., and the mastic curve was 1221000000.

After discharge she was not seen until June 1 at which time the facial palsy had entirely cleared, there were no symptoms, neurologic or mucocutaneous lesions were absent, and the Eagle titer was 32 units. Seven weeks later, July 6, 1944, the Eagle titer was 8 units. The spinal fluid then was normal except for a positive Wassermann reaction with 0.6 cc. The patient was last seen on August 24, 1944, at which time she was asymptomatic and the spinal fluid was normal. Table XI illustrates the course.

Case 10. (JHH 312453) A 19 year old white male referred to us by Dr. Angus L. MacLean complained of severe diminished vision of one month's duration. There had been poor visual acuity in the left eye since early childhood related, according to the patient, to an operation performed on the eye at the age of 6. The nature of this operation is unknown though it seems likely that it was an attempt to correct a strabismus. His general health had been excellent except for this finding. In October, 1943, he was classified 4F by Selective Service because of defective vision.

There was no history of any venereal disease and specifically no knowledge of early syphilis though sexual activity was promiscuous for the year prior to admission. The onset of the present illness was abrupt with severe loss of vision about one month before entry. This improved temporarily at the end of two weeks only to become worse again.

Upon admission to the hospital on February 26, 1944, examination revealed a healed annular penile lesion and a moderate general enlargement of the lymph nodes. One *T. pallidum* was seen upon darkfield examination of material removed from one of the lymph nodes. The visual acuity was considerably reduced being: R.E. 4/200, L.E. 20/200. Fundiscopic examination revealed bilateral swelling of the optic discs and surrounding retinal edema. The blood Eagle flocculation test was 64 units. The spinal fluid contained 2 cells and 22 mgm. of protein per 100 cc. The Wassermann reaction was positive with 0.2 cc. and the mastic curve was 1100000000.

Treatment with sodium penicillin was started on February 28, 1944. The drug was given intramuscularly every three hours for eleven days. A Herxheimer reaction was feared so initial doses consisted of only 1000 Oxford units given every three hours for eight doses. No reaction was observed, and larger doses were employed so that the total amount given in 11 days amounted to 2,008,000 Oxford units.

TABLE IX

The Course under Penicillin Therapy of Case 9

DATE	DAY AFTER PENICILLIN TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
2/ 7/44	0	Penicillin 60,000 units	256	Normal	Early syphilis DF+ genital lesions
2/24/44	16		128		Lesions healed
3/16/44	37		64		Asymptomatic
5/ 4/44	86		32	48 cells 40 mgm. % protein WaR. pos. with 0.2 cc. Mastic 2431000000	DF+ genital lesion; headache, dizziness Left VII nerve paralysis Hearing loss: rt. 30% lt. 15%
	Day after second course				
5/ 6/44	0–8	Penicillin 1,200,000 units			
5/14/44	9		64	14 cells 33 mgm. % protein WaR. pos. with 0.4 cc. Mastic 1221000000	Symptomatic improvement; left VII improved
6/ 1/44	27		32	0 cells 19 mgm. % protein WaR. pos. with 0.8 cc. Mastic 1100000000	Left VII normal No symptoms
7/ 6/44	62		8	5 cells 19 mgm. % protein WaR. pos. with 0.6 cc. Mastic 0000000000	Hearing normal
8/24/44	111		8	Normal	Asymptomatic

Improvement in vision began on the fifth day and at the end of one week he was able to read small print. At the end of the 9th day of treatment the visual acuity was: R.E. 20/50, L.E. 25/200. The swelling of the optic discs and retinal edema had likewise improved and was completely gone by the 18th day from onset of treatment. Visual acuity in the right eye returned to normal by the 46th day, and there has been no further improvement in the vision of either eye since that time. The residual poor vision on the left is explainable on the basis of amblyopia exanopsia. The blood Eagle flocculation titre has shown a steady fall so that when last examined on September 8, 1944, it was 8 units. Spinal fluid abnormalities have persisted as is shown in Table X.

DISCUSSION

The results obtained in these ten patients are briefly summarized in Table XI.

In all patients except three (Cases 5, 8 and 10), infection with syphilis was recent—less than 8 months' duration. In case 8 the duration of infection was 20 months. In Case 5 there was no history permitting the duration to be defined; the collateral evidence of the patient's race, sex, and age suggests that it may have been of some years standing. In Case 10 the onset of infection cannot be defined, but was probably recent.

Four patients (Cases 1, 5, 6 and 10) had had no previous antisyphilitic treatment of any sort; and two of these (Cases 1 and 6) had associated mucocutaneous lesions of early syphilis. The other six patients all had received varying amounts of antisyphilitic treatment given for early syphilis. This previous treatment was grossly inadequate in all except Case 8. These six cases fall into the category of neurorecurrence. It is of particular interest that in one of them (Case 9), the initial treatment for early syphilis had been a course of 60,000 units of penicillin, a dosage now demonstrated (2) to be inadequate.

The clinical manifestations were typical of acute syphilitic meningitis in all, including headache (all cases), stiff neck (3 cases), cranial nerve palsies (5 cases), papilledema (2 cases), convulsions, drowsiness and stupor (2 cases).

The dose of penicillin employed ranged from 600,000 to 4,000,000 units; the duration of treatment was seven and one half to eleven days. These dosages and time intervals are not suggested as the optimum and were chosen because of similar treatment schedules employed at

TABLE X

The Course under Penicillin Therapy in Case 10

DATE	DAY AFTER TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
Oct. '43					Rejected Selective Service because of poor vision L.E.
1/13/44					Sudden almost complete loss of vision
1/25/44			+	4 cells 40 mgm. % protein WaR. pos. with 0.4 cc. Mastic 1100000000	R.E.—20/100 L.E.—20/100 Bilateral neuroretinitis
2/26/44			64	2 cells 22 mgm. % protein WaR. pos. with 0.2 cc. Mastic 1100000000	R.E.—4/200 L.E.—20/200 DF+ lymph node puncture
2/28/44		Penicillin course begun total dosage 2,008,000 units in 11 days			
3/ 1/44	3				R.E.—4/200 L.E.—20/200
3/ 3/44	5				R.E.—20/200 L.E.—25/200
3/ 5/44	7		128		R.E.—20/70 L.E.—25/200
3/ 7/44	9				Vastly improved R.E.—20/50; L.E.—25/200
3/10/44	12			4 cells 37 mgm. % protein WaR. pos. with0.6 cc. Mastic 1222000000	Discharged Greatly improved

TABLE X—*Continued*

DATE	DAY AFTER TREATMENT	TREATMENT	BLOOD STS, TITER IN UNITS	SPINAL FLUID	REMARKS
3/16/44	18		64		Optic discs and retina normal R.E.—20/50; L.E.—20/200
3/27/44	29		32		Further improved
4/13/44	46		16		R.E.—20/20; L.E.—20/200
4/27/44	60		12		
9/ 8/44	193		8	47 cells 40 mgm. % protein WaR. pos. with 0.6 cc. Mastic 5431000000	R.E.—20/30; L.E.—20/200

the time in the treatment of uncomplicated early syphilis. In general, the earlier patients treated received the smaller dose of 600,000 units. Later, as information accumulated as to the direct relationship between total dosage and relapse in early syphilis (1, 2) and the comparative ineffectiveness of doses less than 1,200,000 units, larger doses have been employed in the meningitis cases.

As previously stated, the route of administration was intramuscular in all these cases. Before this work was begun, we were of course aware of the observations of Keefer and Rammelkamp (4) that penicillin injected intravenously or intramuscularly did not appear in the cerebrospinal fluid in any significant quantity. Furthermore, McDermott, Eagle, and Nelson (5) were unable to demonstate penicillin in the cerebrospinal fluid in patients with early syphilis, late neurosyphilis, and tuberculous meningitis. Nevertheless, in neurosyphilis there is reason to believe that the effectiveness of a chemotherapeutic agent does not necessarily depend on the ability of that agent to penetrate the cerebrospinal fluid, but instead, on the basis of penetrability into the diseased tissues (meninges, blood vessels, parenchyma) (6). The effectiveness of intramuscular treatment in early neurosyphilis and the lack of

TABLE XI

Summary of Clinical, Seriologic and Spinal Fluid Result in All Patients

CASE	DURATION SYPHILIS	PREVIOUS TREATMENT	NEUROLOGICAL MANIFESTATIONS	PENICILLIN THERAPY		DAYS OF OBSERVATION AFTER PENICILLIN	SYMPTOMATIC RESPONSE	BLOOD STS TITER UNITS		CEREBROSPINAL FLUID							
										At start of treatment				Last Observation			
				Tot. Units	Days of Rx.			At start of Rx.	Last Obs.	Cells	Prot. mg. %	WaR.	Mastic	Cells	Prot. mgm. %	WaR.	Mastic
1	few mos.	none	Rt. N. VII, bilat. N. VIII, headache, vertigo	1,240,000	8	310	Complete relief	4	0	53	25	4 with 0.4 cc.	0000000000	0	25	0 with 1.0 cc.	0000000000
2	4 mos.+	6 × As.	Headache, stiff neck, papilledema	640,000	8	263	Complete relief	16	D	1280	100	4 with 0.05 cc.	5555430000	12	22	4 with 0.6 cc.	2110000000
3	7 mos.	2-25 × As & Bi	Headache, stiff neck, convulsions, stupor	600,000	7½	167	Complete relief	0	0	102	105	4 with 0.05 cc.	4321000000	4	33	0 with 1.0 cc.	0000000000
4	4 mos.	6 × Maph 2 × Bi	Headache, convulsions, stupor	600,000	7½	176	Complete relief	2	0	70	25	4 with 0.4 cc.	1100000000	0	22	0 with 1.0 cc.	0000000000
5	?	None	Headache; Rt. VI, VII and VIII paralysis	1,200,000	7½	104	Improved; slight residual cranial nerve weakness	2	0	70	118	4 with 0.2 cc.	5554300000	8	51	4 with 0.6 cc.	5554300000
6	3 mos.	None	Headache; left VII palsy	1,200,000	7½	121	Complete relief	16	4	1450	158	4 with 0.2 cc.	5555554000	7	26	4 with 0.8 cc.	4431000000
7	5 mos.	3 × Maph	Headache, stiff neck, rt. VII and bilateral VIII paralysis	600,000	7½	133	Complete relief	D	0	57	37	4 with 0.2 cc.	5543110000	0	25	0 with 1.0 cc.	0000000000
8	20 mos.	36 × As. 20 × Bi.	Headache, vertigo, "nervousness"	4,000,000	10	139	Complete relief	6	1	225	50	4 with 0.5 cc.	5554321000	8	40	4 with 0.4 cc.	4321000000
9	4 mos.	60.000 u. penicillin	Left VII, bilateral N. VIII palsy	1,200,000	7½	111	Complete relief	32	8	48	40	4 with 0.2 cc.	2432100000	4	20	0 with 1.0 cc.	0000000000
10	?	None	Bilateral neuroretinitis	2,008,000	11	193	Complete relief	64	8	2	40	4 with 0.2 cc.	1100000000	47	40	4 with 0.6 cc.	5431000000

necessity for use of the intrathecal route, is borne out by the results presented here.

RESULTS OF TREATMENT

To the date of writing (September 15, 1944), the patients treated have been followed for 104 to 310 days after the start of penicillin therapy (8 of the 10 for 4 months or longer). No clinical relapses have so far been observed, and re-treatment, whether with penicillin or by some other method, has not yet been required. (One patient, Case 10, has shown a relapse in the spinal fluid findings after 193 days and is to be retreated.) Judging from the observed incidence of relapse in patients with uncomplicated early syphilis treated with 600,000 units (relapse rate about 20–25 per cent) (2), relapse in some of these patients with acute syphilitic meningitis is certainly to be expected.

The immediate symptomatic result has been dramatically favorable in all 10 cases. Headache and stiff neck have disappeared in 24–48 hours. Cranial nerve paralyses have disappeared completely in all cases except one (Case 5), in which slight residuals remained after 98 days. Four patients had nerve deafness (Cases 1, 5, 6 and 9) and in three, improvement was gradual, requiring respectively 110, 98, and 139 days for return to complete or nearly complete normal as objectively measured by audiometer tests. One patient has shown no improvement in deafness during 104 days of observation. Two patients (Cases 3 and 4) had no further convulsions after penicillin was started.

Three patients (Cases 3, 5 and 8) had mild febrile Herxheimer reactions (highest temperature 102.4°F.), but this did not occur in the other seven cases. In none was there any clinical exacerbation suggesting therapeutic shock, nor were there any other reactions from treatment.

One patient (Case 3) had a doubtful blood serologic test for syphilis before treatment, which became and remained negative thereafter. Three cases (Cases 4, 5 and 7) with low titer tests (each 2 units) at the start of treatment have become seronegative. In the other 7 cases the serologic titer is falling toward negative in all.

The effect upon the spinal fluid is even more remarkable. The original cell counts before treatment varied from 42 to 1450. In all patients the cerebrospinal fluid was retested on the day of or immediately following the cessation of penicillin therapy, and in all, an abrupt fall

in cell count was observable even at this early period. At the last observation, cell counts were normal (assuming a count of 10 cells or less per cubic millimeter to be normal) in 8. In one patient (Case 2 whose original cell count was 1280) the cell count was 12, and in only one patient (Case 10) has there been an increase in cell count during observation.

The protein content was abnormally high in nine of the ten cases (range 37–158 mgm. per 100 cc.). In all of these, there was a fall toward normal; in four cases, the protein reached a normal level (25 mgm. per 100 cc. or less).

The Wassermann test of the spinal fluid was positive before penicillin treatment in all cases, varying in titer from 0.05–0.8 cc. In all cases, there was improvement, the extent of which varied with the strength of the original reaction and the duration of observation. Improvement has been slower than with cell count or protein content but so far has been continuous and progressive in all cases. Five patients (Cases 1, 3, 4, 7 and 9) have reverted to negative (with 1.0 cc. of fluid), and these are the cases which have also shown the greatest improvement in the blood serologic tests for syphilis.

The mastic curve, abnormal in all patients except Case 1 and ranging in type from relatively moderate to extreme zone I (paretic type), has likewise improved in all except one (Case 10), and has become negative in five.

So far as can be determined from this small number of cases and from the relatively short time in which they have been followed, there is no relationshipship between total dosage of penicillin and the clinical or eventual serologic result, or in the rapidity with which serologic improvement occurs.

SCHEDULE ADVISED FOR THE TREATMENT OF ACUTE SYPHILITIC MENINGITIS

In spite of the observations in the preceding paragraphs, it is believed, on the basis of the results accumulating in uncomplicated early syphilis (2), that patients with acute syphilitic meningitis should receive a total of 2,000,000 to 3,000,000 Oxford units of penicillin administered by the intramuscular route every 3–4 hours day and night, over a minimum period of 8–16 days. There appears to be no reason to

suggest intravenous or intrathecal administration; the former is probably relatively ineffective (2), the latter unnecessary. All patients with acute syphilitic meningitis treated with penicillin should be closely followed with clinical re-examinations (especially for the detection of infectious mucocutaneous or neurorelapse) and with laboratory studies of blood and spinal fluid. These follow-up examinations should be made at least as often as every three months during the first year after treatment, every six months for another 2–3 years, and yearly thereafter. It should not be forgotten that although symptomatic (and often serologic) improvement can be secured in acute syphilitic meningitis with almost any form of modern chemotherapy or fever therapy, the eventual prognosis of this condition in terms of development of late neurosyphilis, especially paresis and tabes, is grave (6) (7).

SUMMARY

1. In ten cases of acute syphilitic meningitis treated by us with penicillin, the immediate results (98 to 310 days after treatment) are excellent, both from clinical and laboratory standpoints.

2. Although penicillin does not appear in the cerbrospinal fluid even after frequent intramuscular administration, the drug is effective in acute syphilitic meningitis when given by the intramuscular route.

3. None of the ten patients treated has so far developed any evidence of clinical relapse, though one has shown relapse in the spinal fluid findings.

4. The total amount of penicillin administered to any one patient varied from 600,000 to 4,000,000 units; the duration of treatment varied from 7½ to 11 days.

5. On the basis of present information, the treatment schedule advised for acute syphilitic meningitis is a total dose of 2,000,000 to 3,000,000 Oxford units of penicillin administered every 3–4 hours day and night for from 8–16 days.

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THE TRYPTOPHANE REQUIREMENT OF MAN AS DETERMINED BY NITROGEN BALANCE AND BY EXCRETION OF TRYPTOPHANE IN URINE[1]

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In a previous study we have shown that tryptophane is a human dietary essential. Subjects maintained on a tryptophane deficient diet for periods of 2 to 5 weeks promptly went into negative nitrogen balance, nitrogen equilibrium being restored on the addition of tryptophane. A single experiment carried out by Cox, Mueller and Fickas (2) led to this same conclusion, and a recent report by Rose (3) mentions experiments carried out by him in which omission of tryptophane led to negative N-balance. Since these studies furnished no exact information about minimal tryptophane requirements, it was decided to carry out further studies in which a tryptophane deficient diet was supplemented by gradual additions of tryptophane in order to determine the minimal intake that would restore N-equilibrium.

We were further interested in following the excretion of tryptophane in the urine in subjects on a tryptophane deficient diet to see if this could be correlated with the deficiency and could be employed as a diagnostic criterion of tryptophane deficiency.

Our experimental technique was essentially that described in our previous report (1). The experimental diet consisted of fats, starches and certain vegetables and fruits selected for their low protein content which provided approximately 10% of the daily nitrogen requirement. The remaining 90% was supplied as an acid hydrolysate of casein supplemented with 1% *l*-cystine. In the present experiments the caloric intake as well as the N-intake was adjusted to the weight of the individual: 38–43 calories per kg. per day and 0.1 g. per kg. per day, respectively.

The subjects of the study were 2 normal adult males who pursued a

[1] This study was carried out under grants received from the Rockefeller Foundation, Nutrition Foundation, Inc., and Merck & Co.

sedentary life during the period of the experiment. Preceding the onset of the experiment subject #9 had been on a low protein diet for 2 days, whereas subject #8 had been on his customary diet. A control period of 6 days' duration was first carried out in which both subjects received the tryptophane deficient diet with a supplement (1.5% of the protein moiety) of *l*-tryptophane. This was followed by a period of 36 days on the deficient diet without any supplementation. *l*-Tryptophane was then added to the diet, first at a concentration of 0.5% of the protein moiety, which was later increased at 3 day intervals to 1.0% and 1.5%. The tryptophane supplement at these 3 levels of intake amounted to 3.1, 6.3 and 9.4 mg. per kilo per day.

Nitrogen balances were determined for 3 day periods throughout the study. Tryptophane in the urine, determined by the procedure of Albanese and Frankston (4), was measured daily and averaged for 3 day periods corresponding to the N-balance periods. It is necessary to make the tryptophane determinations promptly in order to avoid deterioration which occurs if the specimen is allowed to stand for days.

RESULTS

The subjects remained free from clinical symptoms, nor did their blood proteins exhibit changes such as we have observed in rats (5) on a comparatively long period of tryptophane deficiency. The data on body weight, N-balance and urinary tryptophane excretion are shown in Fig. 1. They confirm previous observations on the indispensability of tryptophane for man, loss of weight and negative N-balance resulting from a deficiency of this amino acid. The lowest level of tryptophane supplementation failed to influence the nitrogen balance appreciably. Subject #8 exhibited positive N-balance when the tryptophane supplement was increased to 1% of the protein moiety (6.3 mg. tryptophane per kg.), but subject #9 showed such a response only when the intake was further increased to 1.5% of the protein intake (9.4 mg. tryptophane per kg.). These observations point to a daily tryptophane requirement between 6.0 and 9.0 mg. per kilogram.

Of particular interest is the excretion of tryptophane in the urine. In the control periods the subjects excreted from 250 to 400 mg.

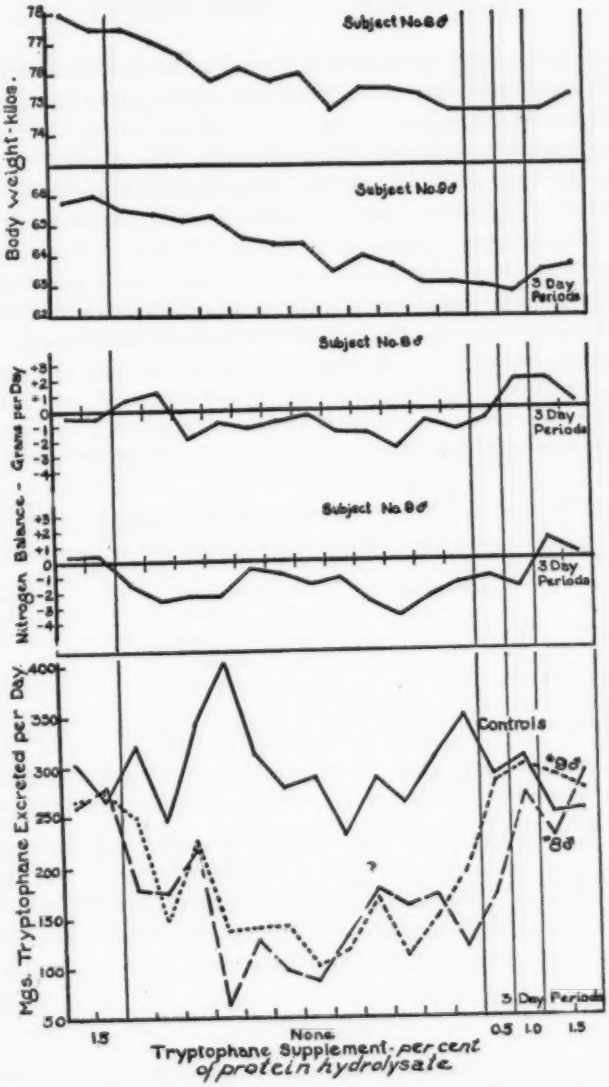

FIG. 1. BODY WEIGHT, NITROGEN BALANCE AND EXCRETION OF TRYPTOPHANE IN THE URINE OF 2 SUBJECTS ON A TRYPTOPHANE DEFICIENT DIET

tryptophane per day, as we have found to be the case in subjects on a normal diet. With the institution of the tryptophane deficient diet the urinary tryptophane fell sharply, remaining below 250 mg. per day (and usually well below this) as long as the deficient diet was continued. One of the two subjects responded to the lowest level of tryptophane supplementation, the other at the second level, by a return of tryptophane excretion to the normal value. The reduction in tryptophane excretion caused by the deficiency is all the more striking when compared with that of a group of experimental subjects studied simultaneously who received an identical basal diet but were given casein hydrolysates deficient in amino acids other than trypto-

TABLE 1

The Tryptophane Requirement of Man as Reported by Various Investigators

INVESTIGATORS	SUBJECTS	TOTAL INTAKE	PROTEIN COMPONENT OF DIET	TRYPTOPHANE REQUIREMENTS	METHOD OF ASSAY
		Cal/kg/day		*mg/kg/day*	
Present	adult males	38–43	hydrolyzed casein	3.0–6.0	tryptophane excretion
Present	" "	38–43	hydrolyzed casein	6.0–9.0	N-balance
Martin and Robinson (6)	" "	57	milk	9.3	N-balance
Rose and MacLeod (7)	" females	40	milk	6.2	N-balance
Macy (8)	adolescents	58.5	mixed	19.8	calculation

phane (four subjects on a diet deficient in cystine and methionine; three subjects on a diet deficient in histidine). These other experimental subjects, all of whom received adequate tryptophane, continued to excrete tryptophane in normal quantities. An average curve of their excretion is plotted under the caption "controls" in Fig. 1.

COMMENT

Attention may be called to the fact that the tryptophane excretion value responded more promptly to the administration of tryptophane than did the nitrogen balance. It is not possible to say which of these two criteria—tryptophane excretion or N-balance—furnishes

the more accurate data on the minimum tryptophane requirement in these experiments. It is conceivable that the prompt excretion of tryptophane after supplementation was started is in part due to a loss of tolerance. On the other hand, the fact that in subject #8 this rise did not follow immediately upon supplementation points against this explanation. There is known to be a lag in establishing N-equilibrium which may account for the fact that this occurred at a higher level of tryptophane intake than the change in urinary tryptophane. Possibly if the supplementation periods had been longer, a restoration of N-equilibrium would have occurred at a lower level. We had originally planned to have considerably longer supplementation periods, but the induction of the 2 experimental subjects into the military forces made it necessary to curtail our experiments somewhat. We have, however, calculated the daily tryptophane requirements from both sets of our data. These are shown in Table 1 together with the estimates of other investigators that have been published. It appears that the tryptophane requirement is not as great as has been estimated.

SUMMARY

The human requirement for tryptophane has been studied by the stepwise addition of *l*-tryptophane to a tryptophane deficient diet. Restoration of nitrogen equilibrium occurred with a supplement of 6.0 to 9.0 mg. tryptophane per kg. per day.

The excretion of tryptophane in the urine was found to decrease sharply on a tryptophane deficient diet, remaining below 250 mg. per day. A tryptophane supplement of 3.0 to 6.0 mg. per kg. sufficed to restore this to normal levels.

The tryptophane requirement, as determined by these two criteria, has been compared with other values in the literature.

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CHORIONIC GONADOTROPIN AND PREGNANEDIOL VALUES IN NORMAL PREGNANCY

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Data will be presented on the production of chorionic gonadotropin and excretion of pregnanediol in normal human pregnancy. Since the demonstration of the existence of the various hormones in pregnancy, these substances have been invoked frequently as causal factors in reproductive failures and in various pathological processes. In spite of this interest in presumed relationship to abnormal states, there is rather meager data on the quantitative occurrence and individual variations in these substances in normal pregnancies.

A study has been made of the chorionic gonadotropin in the serum of 24 women and of the pregnanediol excretion in the urine of 18 of the same women. All of these women had entirely normal pregnancies and each delivered a normal child uneventfully. Two additional cases having twin pregnancies were studied. Determinations were repeated at intervals throughout each pregnancy so that both general trends and individual variations would be brought out. Such data may serve as a reference standard for evaluation of pathological conditions.

Reported observations on chorionic gonadotropin have remained limited because of the tedious bio-assay involved. Summation of data of different workers is almost impossible due to diversity of assay techniques and standards. A general curve for urinary excretion of chorionic gonadotropin throughout pregnancy was established with the study of 5 cases by Browne and Venning (1) and 6 cases by Evans, Kohl, and Wonder (2). They found significant amounts of gonadotropin at about 40 days after the last menstrual period, a rapid rise to a peak at 60–65 days, a decline to a relatively low level by 120 days with continuation of this general level to term. Others have amplified this data with a few additional cases (3, 4).

Chorionic gonadotropin in the serum during pregnancy was studied early by Kennedy (5) who reported a progressive rise from early to late pregnancy. This increasing concentration was not corroborated by Rowlands and Boycott (6) and Rakoff (7), whose composite charts of individual determinations on a number of women at various stages of pregnancy resemble the urinary excretion curve.

They showed a peak in gonadotropin at 8–12 weeks with a decline to a low level at 16–20 weeks which was maintained to term. Concurrent studies on urine and serum are few but indicate that the values parallel each other in general and are of the same order of magnitude (2, 4, 8, 9, 10).

The preganediol excretion throughout normal pregnancy was first reported by Venning and Browne (11) who gave a composite curve for a series of 8 normal pregnancies. Additional composite curves have been given subsequently by Stover & Pratt (12), Bachman et al. (13) & Tein (14), and individual case curves by Hain (15), Cope (16), Smith & Smith (17), and Thompson, Mussleman, & Greer (18). In 1942, Hain (19) published her wide experience with pregnanediol determinations in a number of normal and abnormal pregnancies. All of the authors have used the method described by Venning and Browne with minor modifications.

METHODS

Chorionic Gonadotropin: Determinations of chorionic gonadotropin were made on blood serum. This is more reliable than urine as an index of actual gonadotropin production. The concentration in single urine specimens varies with fluid output and 24-hour specimens suffer some loss due to deterioration during collection. Blood specimens were drawn as frequently as practicable, especially early in pregnancy when rapid changes in gonadotropin level take place. The serum was prepared and assayed according to a method previously reported (20). Immature rats (21–23 days old) of standard weight were injected six times over a two day period with autopsy at 72 hours after the initial injection, and uterine weight increase was used as the endpoint. Chorionic gonadotropin values have been expressed in international units as the serum dose-response curve has been standardized against international standard chorionic gonadotropin. (In our colony one I.U. produces an average uterine weight response of 79 mg.) This method, using 3 or more animals at a dose level, has been found more sensitive and accurate than assays using corpora lutea or other endpoints. Sera were prepared in dry form in duplicate so that assays could be repeated with fresh solutions whenever there was not close agreement among all of the animals.

Pregnanediol: The pregnanediol method used was that described by Astwood and Jones (21), with several minor modifications. This method was devised for speed, ease, and accuracy but should be performed by a well trained chemist who understands the general problems involved in sterol chemistry.

The urine specimens were collected with 2 grams of sulfanilamide powder, shaken often and kept as cool as possible. Total 24 hour volumes were obtained and the entire specimen or a suitable aliquot, depending on the amount of pregnanediol which the urine was expected to contain, extracted. A suitable volume for extraction was considered one which contained not more than approximately 20 mg. of pregnanediol.

The urine is placed in a flask and one twentieth of the volume, or not less than

50 ml., of toluene added. A wide bore reflux condenser is attached with a cork-stopper or preferably a ground glass joint and the urine heated to boiling. Ten volumes per cent HCl (sp. gr. 1.19) are added slowly and boiling continued for 15 minutes. The flask is cautiously removed from the flame and allowed to cool until it can be handled comfortably (violent boiling may occur if the hot mixture is shaken). It has been found that the pregnanediol extraction is facilitated if extractions are carried out while the urine toluene mixture is still warm. The urine is therefore transferred to a separatory funnel as soon as possible. The toluene layer, together with the urine toluene emulsion layer, is removed and the urine re-extracted twice with $\frac{1}{5}$ the volume of toluene or not less than 25 ml. The combined toluene and emulsion extracts are filtered through a Buchner funnel with gentle suction and the residue thoroughly washed with small amounts of hot toluene. If suction is not excessive the emulsion can be completely broken by this method. The filtrate is poured into a separatory funnel, the urine layer is discarded, and the toluene extract washed three times with 10 ml. of water. The toluene is transferred to a dry, round bottom, short neck, 24/40 tapered joint, boiling flask and connected to a standard vacuum still by means of a 90 degree connecting tube. The toluene is then distilled off under vacuum on a steam bath until no trace of water can be seen in the solution. Two ml. of a 10% solution of metallic sodium in 95% ethyl alcohol are added and distillation is continued until the volume of toluene is reduced to approximately 50 ml. The colloidal or granular precipitate which forms is filtered off and the purified toluene extract is collected in a 200 ml. round bottom boiling flask. The original flask and precipitate are thoroughly washed with three successive amounts of hot toluene. The filtrate should be clear and free from all traces of a pink or brown color, otherwise the above step must be repeated. The clear, greenish yellow filtrate is then distilled to dryness under vacuum on the steam bath. The white residue is taken up completely in 10 ml. of hot 95% ethyl alcohol. The pregnanediol is slowly precipitated from the alcoholic solution by the addition of 40 ml. of hot aqueous 0.1 N sodium hydroxide. The stoppered flask is allowed to stand until cool then placed in the refrigerator over night to assure complete precipitation. The crystalline pregnanediol is collected by filtration, washed with water and petroleum ether (B.P. 60–70 degrees C.), and redissolved in hot ethyl alcohol. A second precipitation is carried out in like manner using 10 ml. of hot ethyl alcohol and 40 ml. of hot distilled water in place of the NaOH. If the pregnanediol precipitate is not crystalline and almost colorless a further precipitation from aqueous alcohol is made. The purified substance is collected by filtration, washed with water and redissolved in hot alcohol. The alcohol volume is reduced to between 5 and 10 ml. and allowed to cool. The cool alcoholic solution is transferred to a suitable small tared vial or weighing bottle and the flask and funnel carefully washed with small amounts of hot alcohol. The sample is dried to constant weight in an oven at 80 degrees C. and weighed. A melting point is taken on the final product and considered satisfactory if it is not below 220 degrees C.

DISCUSSION

Values for chorionic gonadotropin in international units per liter of serum are plotted for days of pregnancy calculating from day one of the last menstrual period. Typical curves for several individual pregnancies which were studied at frequent intervals are shown in charts 5A to 5F. A composite of the values for all of the cases (Chart

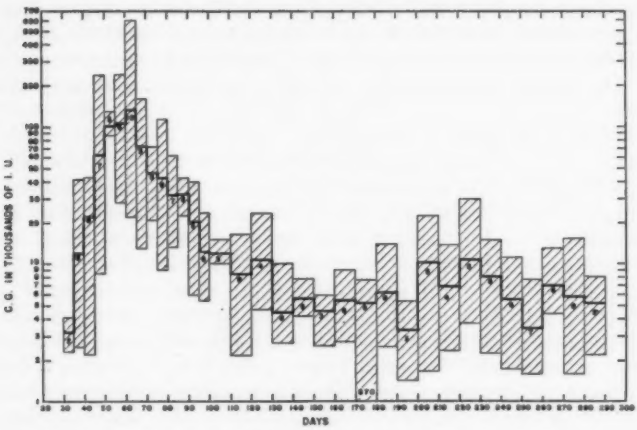

CHART 1. SERUM CHORIONIC GONADOTROPIN THROUGHOUT NORMAL PREGNANCY IN 24 WOMEN

Individual values are given in Table 1. Gonadotropin in I.U. is plotted against duration of pregnancy in days, counting from day one of the last menstrual period. Heavy black line shows average gondatropin level. Cross-hatched area indicates extreme variation for the period with the number of determinations in each. All charts are plotted on semilogarithmic graphs as these demonstrate percentage deviation better and are more adaptable to a wide range than linear scales.

1) illustrates the average curve and also the range of maximum and minimum values for each 5 or 10 day period throughout pregnancy. The shorter interval is used in the early period because of the rapid changes occurring at that time.

The curve rises sharply reaching a peak between 50 and 65 days. Occasionally the peak may occur even earlier as in case 5E which was followed at frequent intervals and reached a maximum at 45 days.

In some cases, the true peak may have been missed when specimens were taken at weekly or longer intervals. The apparent variation in the time at which the peak occurs may be due in part to the fact that the "day of pregnancy" is calculated, of necessity, from the last menstrual period rather than the ovulation date, hence the actual age of the pregnancy may differ several days from case to case. Charts 5A and 5E show very early peaks and these individuals are known to have consistently rather short cycles with evidence of early ovulation. Another factor may be involved in determining both the time of the peak and the level of gonadotropin production attained in early pregnancy. The trophoblast may vary in rate of growth and secretion in different pregnancies. How close correlation there is between secretion of gonadotropin and functional adequacy of the trophoblast cannot be stated with certainty but Chart 1 demonstrates that considerable latitude is compatible with normal pregnancy. In some cases the peak did not exceed 70,000 I.U. per liter, in several it was over 200,000, and in one it reached 600,000. It is clear that diagnosis of pathological pregnancy, e.g. hydatid mole, cannot be made from isolated high observations at this stage of pregnancy. On the other hand, pregnancies which show repeated levels below the minimum range of chart 1, usually do not progress normally.

The decline in gonadotropin is more gradual than the rise, but by day 100 the level drops to 10–12,000 I.U. There is some further decrease in the next 30 days to an average level of 5–6,000 I.U. From this time to the end of pregnancy, the gonadotropin remains low; most of the determinations are below 10,000 I.U. and only four times were values over 15,000 found after day 120. Each of these cases continued to term normally. The suggestion of a slight secondary elevation between 200–230 days is probably not important. The apparent variations for values in late pregnancy are actually not great but are accentuated by the semilogarithmic scale.

The significance of the rapid decrease in gonadotropin in the fourth month and after is not clear but it may mirror a functional decline and ultimate disappearance of most of the Langerhans' cells. Tissue culture studies by Jones, Gey, and Gey (22) showed that these cells are probably the source of chorionic gonadotropin. If the function of this substance is the stimulation of corpus luteum activity, as has

been suggested, it is no longer necessary after placental progesterone production is well established. Its persistence after this time may have little significance; this may be implied by such cases as 5C and 5D which had very low gonadotropin in the late months of pregnancy yet continued uneventfully to term. It is of interest that some other primates apparently eliminate chorionic gonadotropin production entirely before the end of gestation. In the chimpanzee it disappears between days 100–160 (23), and in the monkey is not detectable later than day 30 after ovulation (24). This may be correlated with early decrease of Langerhans' cells in these species. Early chimpanzee placentae have not been studied but a gorilla placenta of 4 months showed few Langerhans' cells (25). The macaque placenta has been closely studied through the first 35 days after ovulation and has fewer Langerhans' cells at this stage than earlier, though some are still present (26). Though data are fragmentary, there is indication of a progression in persistence of the cytotrophoblast from platyrrhine monkeys through catarrhine monkeys and anthropoids to the human (25).

The amount of free pregnanediol excreted throughout pregnancy has been calculated in mgms. per 24 hours and plotted against the duration of pregnancy in days (Chart 2). Eighteen normal pregnancies were assayed in this study and actual values for 17 individual cases can be seen in Table 1. The pregnanediol excretion curve can be roughly divided into two periods: that in which the corpus luteum is the main source of progesterone production and that in which the placenta is probably the most important site of progesterone formation. During the first period, which terminates in the average case somewhere between day 64 and 78, the pregnanediol values are rarely above 20 mgm. or below 6 mgm. per 24 hours. Throughout the remainder of the pregnancy, there is a gradual rise in the pregnanediol excretion curve until about day 250 when the peak values are usually attained. This peak may be maintained as illustrated in Chart 5 (D) and (F), or a decline may be seen as in Chart 5 (A) and (B). The average peak value in the present series was 85 mg. per 24 hours. Values below 40 mg. per 24 hours or above 100 mg. per 24 hours were unusual. In no case was there a complete absence of pregnanediol at any period throughout the pregnancy.

In Chart 2 there is a terminal rise in the pregnanediol curve. By examining Table 1 it can be seen that this is apparent only because those women with gestation periods of 280 days or longer had high pregnanediol values. As the majority of individual curves showed a terminal fall in the pregnanediol curve, it was decided that the values should be plotted against the total duration of the pregnancy to show

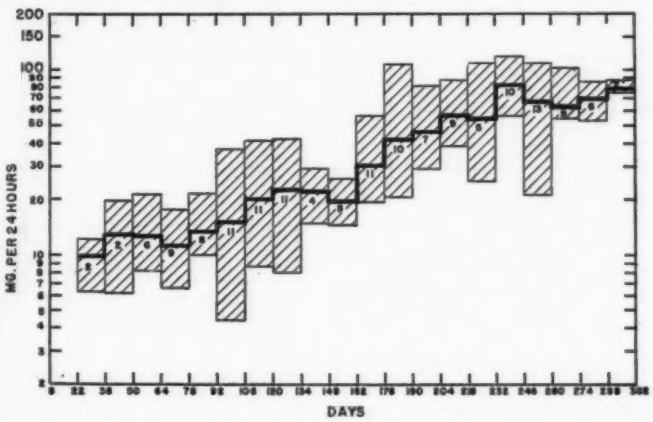

CHART 2. PREGNANEDIOL EXCRETION THROUGHOUT NORMAL GESTATION COMPILED FROM A STUDY OF 18 PREGNANCIES

The actual values are given in Table 1. The amount of free pregnanediol excreted expressed in mg./24 hours is plotted against the duration of pregnancy in days. The average pregnanediol excretion is indicated by the heavy black step line. The extreme variations for the same period are shown by the limits of the cross-hatched area. The number of pregnancies studied, not the number of individual determinations made, has been designated by the numeral in the cross-hatched area.

the curves in relation to the onset of labor. Therefore, the total gestation period was calculated from the date of the last menstrual period and the date of the spontaneous onset of labor. In this series only 17 cases were used as pregnancy was terminated by a Cesarean section because of disproportion in one patient. The pregnanediol values were then plotted against the total duration of the pregnancy as expressed in percentages. The result can be seen on Chart 3. The general features of this theoretical curve are similar to those of

	10		15		20		25		30		35		40		45		
	C.G.	Pregn.	C.G.	Pregn.	C.G.	Pregn.	C.G.	Pregn.	C.G.	Pregn.	C.G.	Pregn.	C.G.	Pregn.	C.G.	Pregn.	C.G.
Case 1							21,500 (d81)		37,500 (d89)	12.6	12,500 (d104) 12,400 (d112)	22.9 27.8	9,200 (d126)	31.9			
Case 2					20,970 (d64)	9.7	14,400 (d84)	11.3			5,700 (d99)	13.1	2,300 (d119)	26.0			
Case 3							18,500 (d76)		13,250 (d83) 12,800 (d90)	2.2 10.0	15,000 (d104)	4.9			3,400 (d132)	24.4	
Case 4					166,000 (d60)	14.3			40,000 (d94)	9.3				18.7 (d127)			6,2 (d1
Case 5													23,660 (d122)				5,9 (d1
Case 6												23.3 (d93)					2,6 (d1
Case 7																42.2 (d129)	
Case 8																14.8 (d136)	
Case 9					16,400 (d60)	8.2 (d63)	46,000 (d74) 59,000 (d81)	9.7	71,200 (d88) 53,500 (d98)	13.4 13.6	29,200 (d108)	15.4	14,300 (d119)	21.2		22.7 (d132)	30,3 (d1
Case 10	4,100 (d29) 10,400 (d36)	12.2	70,800 (d45) 130,000 (d51)	9.6	105,000 (d57) 60,000 (d61) 49,000 (d67)	9.1 8.5 11.0	36,250 (d73)	9.0 (d75)	42,500 (d85) 19,400 (d92)	13.2 12.2	13,100 (d103)	14.7 (d107)	6,700 (d114)	22.9	10,000 (d135)	21.2	
Case 11	2,550 (d35)		26,500 (d43)	19.6	84,000 (d56)	21.2	72,000 (d71)	16.9	34,000 (d85)		10,000 (d102)	37.2		33.2 (d113)			
Case 13			10,600 (d47)			10.6 (d58)		6.6 (d74)		14.3 (d88)		14.4 (d111)				22.0 (d131)	
Case 15					80,000 (d66)		47,000 (d73)	11.7					10,000 (d112)	14.0	2,650 (d133)	10.9	
Case 16			42,000 (d44)	6.2			160,000 (d68)	16.5	60,000 (d82) 22,500 (d89)	22.5 13.2			10,900 (d110)	16.5	5,000 (d132)		
Case 17									23,400 (d90)	10.0					4,700 (d127)	33.6	
Case 18							21,000 (d73)	9.3					11,000 (d114)	41.3			
Case 19		6.3 (d34)	30,000 (d45) 242,000 (d56)	10.9			43,800 (d75)	10.3			9,800 (d105)	11.9	5,200 (d126)	18.2			4,7 (d1

Throughout 17 Normal Pregnancies
n last menstrual period

45			60		65		70		75		80		85		90		95		100
C.G.	Pregn.	C.G.	C.G.	Pregn.	C.G.	Pregn.	C.G.	Pregn.	C.G.	Pregn.	C.G.	P.egn.	C.G.	Pregn.	C.G.	Pregn.	C.G.	Pregn.	Del.
			14,600 (d182)	56.8			14,200 (d207)	45.6			11,120 (d239)	93.6		109.6 (d258)	6,900 (d275)	86.0	8,130 (d289)	88.0	295
																			281
3,400 (d132)	24.4				3,000 (d182)	27.4	3,100 (d196)	38.2									5,250 (d267)	30.9	273
		6,2 (d14	7,000 (d177)	20.6			13,800 (d212)					69.4 (d234)							289
		5,90 (d13	6,200 (d188)	23.0							30,000 (d229)	51.4			13,000 (d262)	36.2			284
		2,60 (d13					6,000 (d171)	56.5								43.0 (d220)			241
	42.2 (d129)		2,650 (d172)	30.2	1,430 (d194)	59.2			4,066 (d223)				1,750 (d246)	22.4	4,400 (d265)	43.4	1,500 (d278)		282
	14.8 (d136)			26.4 (d164)				38.0 (d198)					15,200 (d235)	58.8					273
	22.7 (d132)	30,2 (d1	45.8		9,690 (d190)	42.6	5,330 (d206)	76.4	5,860 (d221)		22,700 (d236)	89.4 126.3 (d242)	3,130 (d249)	89.6	13,770 (d271)	76.0 (d261)		76.4 (d278)	288
000 (135)	21.2		7,090 (d170)		3,460 (d189)					48.8 (d211)	3,750 (d223)		6,650 (d241)	64.5		48.8 (d253)	2,900 (d270)	60.4	
			30.2 (d164)	33.8 (d178)		29.4 (d193)						66.8 (d234)	(d246)	84.8		41.2 (d259)		25.2 (d278)	279
			25.9 (d147)		2,750 (d169)	27.8			7,000 (d184)	100.6	22,660 (d205)	76.0 100.4 (d212)	7,530 (d219)	108.4 108.0 (d226)	8,200 (d233)	108.0 146.2 (d240)	7,800 (d247) 8,500 (d257)	71.4 87.3 (d252) 57.4	257
	22.0 (d131)			4,650 (d186)			2,400 (d207)	52.5	10,000 (d221)		3,060 (d242)		7,700 (d257)	70.5	2,350 (d271)	50.7	2,400 (d285)	77.7 (d278) 66.6	288
650 (133)	10.9		14.7		<2,500 (d189)	37.8			2,360 (d217)	72.4		75.4 (d237)	1,780 (d252)	72.0		68.8 (d259)	2,530 (d273)	83.0 94.0 (d280) 29.3 (d285)	286
000 (132)			23.5 (d159)		870 (d176)	44.7			<1,660 (d208)	39.0					1,500 (d252)	48.7			270
700 (127)	33.6		19.5				5,400 (d196)	81.6								80.2 (d256)	3,900 (d259)	102.6	272
					4,800 (d186)	44.5							9,600 (d239)	108.0	3,000 (d258)	67.2	5,100 (d264)	58.8	275
		4,3 (d14	24.3		35.9 (d189)			48.6 (d210			2,250 (d231) 3,200 (d244)	25.2 56.8		47.6 (d258)		54.8 (d272)		53.6 (d287)	288

Chart 2 with the exception that the terminal portion of the curve now shows a slight fall instead of the rise seen in Chart 2.

When the chorionic gonadotropin values for the same 17 patients were plotted in a similar manner, Chart 4, an interesting correlation was noted. The fall in the chorionic gonadotropin curve had definitely begun between 25 and 30% of the duration of pregnancy. At this same period there was a drop in the pregnanediol curve. Such a fall in the pregnanediol curve is of theoretical interest as it is this period which, we believe, marks the beginning of the major placental formation of progesterone. The fall in the chorionic gonadotropin level may indicate that its function of prolonging the activity of the ovarian corpus luteum is no longer necessary. As can be seen, however, the average chorionic values are maintained at a fairly high level until 35% of the pregnancy, at which time the pregnanediol curve has quite definitely begun to rise. Such an overlap would insure that the ovarian activity be maintained until well after the placenta has assumed the function of progesterone formation. This may also suggest that there is a wide individual variation in the time of placental ovarian adoption. If one uses the entire time spread of the chorionic gonadotropin fall as an indication of ovarian adoption, it may be assumed that this occurs as early as 20% in some pregnancies or as late as 35% in others.

There has been much discussion in the literature about the relationship between the onset of labor and the fall in the progesterone level, as measured by pregnanediol excretion. A study of our cases seems to indicate that, although this is probably a contributing factor, it may not be the absolute cause of the initiation of labor. The compilation of curves, Chart 3, shows a slight drop in the pregnanediol curve after 90% of the duration of pregnancy. This drop however represents only a fall of approximately 15 mg. (Table 1). Chart 5, curves (A), (B), and (D) show dramatic falls in the pregnanediol output before delivery. In each case values were obtained within 24 hours of labor. Chart 5 (F), however, shows a drop of only 20 mg., although this specimen was obtained 24 hours prior to the onset of labor. Falls of equally dramatic appearance can occur without signs or symptoms of imminent labor as demonstrated by Chart 5 (A) and (B). Perhaps this indicates that there is a critical level for each

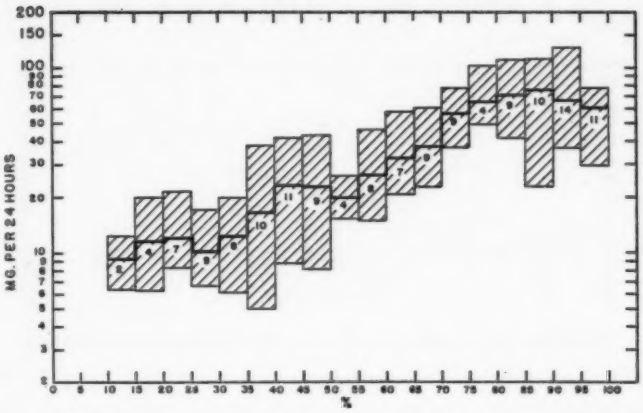

CHART 3. PREGNANEDIOL EXCRETION CURVE ACCORDING TO THE TOTAL GESTATION PERIOD RATHER THAN THE DURATION IN ACTUAL DAYS

The duration of pregnancy expressed in percentage is calculated from the known date of delivery and the date of the last menstrual period. This is a theoretical curve but approximates the appearance of the pregnanediol curve plotted for any individual pregnancy.

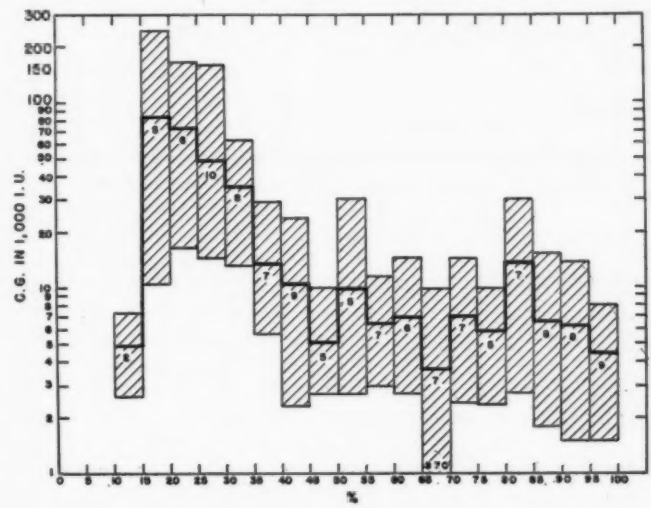

CHART 4. SERUM CHORIONIC GONADOTROPIN PLOTTED AGAINST THE TOTAL DURATION OF PREGNANCY EXPRESSED IN PERCENTAGES, BASED ON A STUDY OF THE SAME 17 CASES SHOWN ON CHART 3

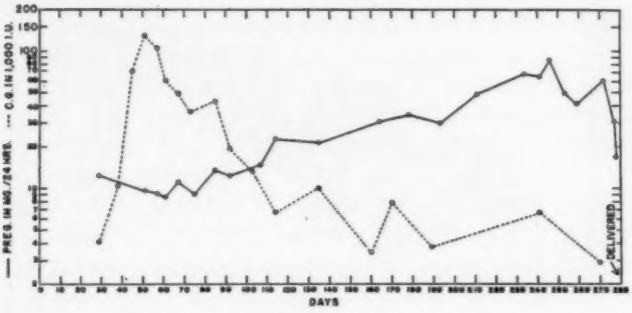

CHART 5A

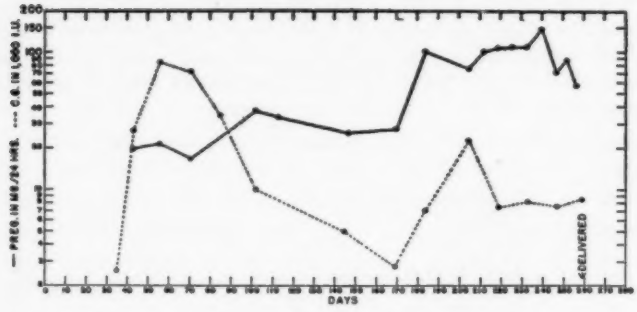

CHART 5B

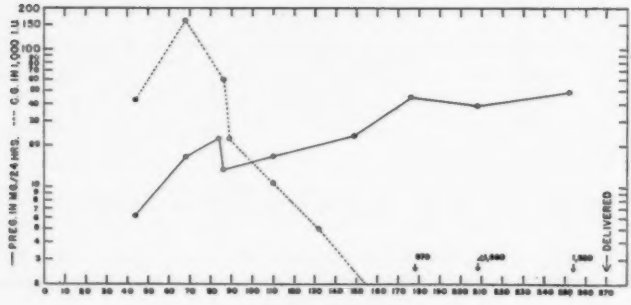

CHART 5C

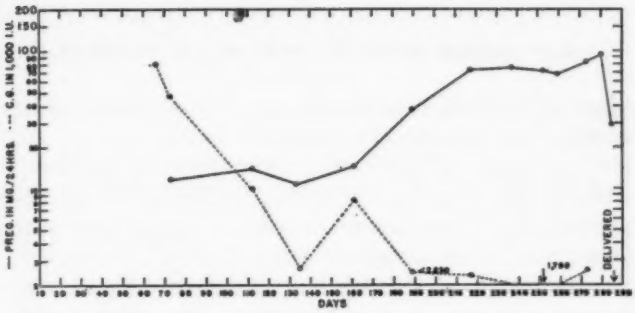

CHART 5D

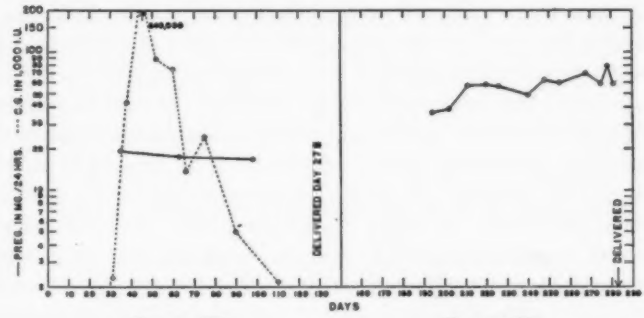

CHART 5E CHART 5F

CHART 5 A, B, C, D, E, and F. PREGNANEDIOL AND CHORIONIC GONADOTROPIN CURVES FOR INDIVIDUAL PREGNANCIES

The unbroken lines indicate the pregnanediol in mgm. per 24 hours, and the interrupted lines the chorionic gonadotropin in thousands of I.U. per liter of blood. Both are plotted against the duration of pregnancy in days.

Chart (*A*) illustrates a pregnancy with relatively low pregnanediol values. The fall at the termination of pregnancy is demonstrated.

Chart (*B*) illustrates a pregnancy with a high pregnanediol level throughout, falling at term. Compare these values with those of the second pregnancy of the same subject (E). The chorionic value of 22,000 at day 205 is high but no evidence of toxemia was observed.

Chart (*C*) illustrates a case with insufficient values near term to demonstrate any drop in the pregnanediol level. These are unusually low chorionic gonadotropin levels in the latter two thirds of pregnancy.

Chart (*D*) shows a sudden drop in the pregnanediol values 24 hours before delivery. Again low chorionic gonadotropin values are seen in late pregnancy.

Chart (*E*) represents the first trimester of the second pregnancy of subject illustrated in Chart (B). As in the previous pregnancy, high pregnanediol values occurred.

Chart (*F*) represents the terminal pregnanediol values in the second pregnancy of subject in Chart (A). These values, although taken weekly and biweekly, do not indicate a monthly cyclic variation in the pregnanediol output. Only a slight drop in pregnanediol is noted before delivery.

individual below which progesterone output cannot fall and be compatible with the continuation of gestation.

It has been suggested that there is a monthly cyclic variation in the pregnanediol output throughout pregnancy similar to that described for the estrogens. If such a variation occurs it is not gross enough to be demonstrable on the composite curve, Chart 3. One patient was studied with weekly values in the last trimester of pregnancy in order to show such a rhythmic pattern if possible, Chart 5 (F). No cyclic variation in the pregnanediol excretion was observed. This fact would seem to be consistent with the theory of the placental adoption of ovarian function. If the placenta is the source of the pregnanediol excreted, there is little reason to believe that any cyclic rhythm, corresponding to the normal cyclic ovarian rhythm, would be manifest.

Multiple Pregnancy: In order to distinguish normal pregnancy from pathological pregnancy endocrinologically, it is important to know the hormonal values in the most common variation of the normal—a multiple pregnancy. It might be expected that, since the placental volume is usually increased in a multiple pregnancy, the hormonal values would be unusually high. Indeed, this seems to be the case for the chorionic gonadotropin values at least. In the present study two cases of twin pregnancy were encountered. One case had only chorionic gonadotropin assays made while the other had both chorionic gonadotropin and pregnanediol determinations. One showed abnormally high chorionic gonadotropin values maintained throughout pregnancy while the other showed only moderately high values in the first half of gestation, the curve later falling within the range of a single pregnancy (Chart 6). The pregnanediol values were also compatible with those found in single pregnancy; this is in contrast to findings in a twin pregnancy reported by Tien (14).

Case 1 delivered mono-zygotic twins weighing 1650 and 1605 grams on day 220, with a placenta weighing 660 grams and measuring 20 x 20 x 2 cm. There was no evidence of toxemia throughout the pregnancy or puerperium. This patient showed consistently high chorionic gonadotropin values during her entire pregnancy. No pregnanediol determinations were made.

Case 2 had had a three year history of endometrial hyperplasia following her first delivery at the age of 19 years. March 7, 1942, she was curetted and the diagnosis of endometrial hyperplasia was made. Cyclic pregneninolone therapy was given over a period of three months, the last course starting May 17. Bleeding ensued May 25, two days following the withdrawal of therapy. She was next

seen July 20. At this time she was having nausea and vomiting and the uterus was soft and slightly enlarged. Pregnancy was suspected and a serum chorionic gonadotropin was taken which confirmed the diagnosis. She delivered di-zygotic twins weighing 1620 and 1489 grams on day 260 after a completely normal preg-

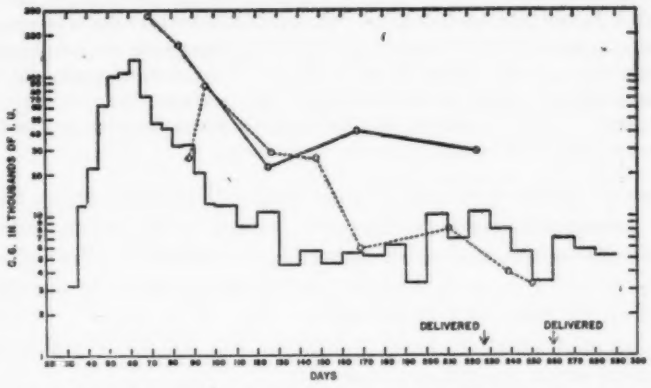

CHART 6. SERUM CHORIONIC GONADOTROPIN IN TWIN PREGNANCIES SHOWN IN RELATION TO THE AVERAGE VALUES FOR SINGLE PREGNANCY

The unbroken line represents Case 1, the interrupted line Case 2. The heavy black step line represents the average values for chorionic gonadotropin throughout pregnancy, based on a study of 24 pregnancies (Chart 1).

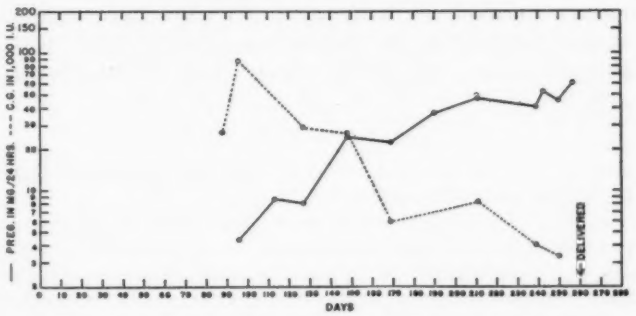

CHART 7. CHORIONIC GONADOTROPIN AND PREGNANEDIOL VALUES IN A DI-ZYGOTIC TWIN PREGNANCY (CASE 2)

nancy. The placentae weighed 327 and 355 grams. The puerperium was uncomplicated. Her chorionic gonadotropin curve was slightly elevated in the first half of pregnancy but the values fell within the range of a normal single pregnancy in the latter half of gestation (Chart 7). Her pregnanediol excretion was never

excessive. This was rather surprising as she probably had two corpora lutea and the pregnanediol values, at least in early pregnancy, might have been expected to be elevated. The case is complicated, of course, by the fact that the woman had a functional ovarian disturbance with deficient corpus luteum function and undetermined ovulation time. The deficiency in corpus luteum function might be illicited to explain the low pregnanediol values. However, in four other cases of pregnancy occurring in patients with histories of endometrial hyperplasia the pregnanediol excretion was normal. As the ovulation time in this case is uncertain, the patient was perhaps as much as 30 days earlier in her pregnancy than the last menstrual period indicates. If this is true the chorionic gonadotropin curve would be set back making it fall exactly within the normal range.

Multiple pregnancies may be associated with elevated serum chorionic gonadotropin values. However, normal levels do not exclude the condition, especially in the latter half of gestation. The volume of the placenta at term does not determine whether the chorionic gonadotropin levels will be excessive.

In our study, the pregnanediol excretion was not above that found in normal single pregnancy (Chart 7).

SUMMARY

Chorionic gonadotropin serum levels and urinary pregnanediol values have been studied throughout the course of normal pregnancy in a group of 18 women. An additional 6 women have been studied for chorionic gonadotropin levels only. Two twin pregnancies have been reported with curves for the chorionic gonadotropin in both and pregnanediol in one. The theoretical significance of the findings is discussed.

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STUDIES ON COMMON PATHOGENIC FUNGI AND ON ACTINOMYCES BOVIS

I. In Vitro Effect of Fatty Acids[1, 2]

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"Mycoban" (trade name of the E. I. duPont de Nemours Company for sodium or calcium propionate) is incorporated in bread dough and cake batter, by many of the large baking companies, to inhibit the growth of molds. As a matter of fact many of the fatty acids and their salts are fungistatic for non-pathogenic fungi (1).

The retarding effect on growth of molds by sodium propionate and calcium propionate in regard to dairy products has been extensively investigated (2, 3, 4, 5). Peck and Rosenfeld (6) have demonstrated that sodium propionate adjusted to pH 5.6 is fungistatic for Trichophyton gypseum, Epidermophyton inguinale and Monilia albicans. More recently we (7) have reported that sodium propionate is fungistatic for all of the common pathogens when the pH is adjusted to 5.5. Clinically (8, 9) sodium propionate prepared in a solution, an ointment and a powder is effective in the treatment of some of the superficial mycotic infections.

The purpose of this paper is to report upon the fungistatic and fungicidal activity in vitro of sodium valerate, sodium caproate, sodium caprylate, sodium caprate and sodium undecylenate. Hoffman et al (1) have reported that the fatty acids of these salts were most effective for non-pathogenic fungi at pH 7.4 and it was our hope that the same would be true for pathogenic fungi. We have felt that optimal activity at pH 7.4 would be desirable, particularly if parenteral administration is to be considered.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

[2] The results of this investigation were presented in part at the meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey, May 8, 1944.

It is our hope by this investigation to discover a fatty acid salt that will prove superior to sodium propionate in the treatment of mycotic infections and it is also our aim to determine whether or not any of these preparations show specific affinity for certain pathogenic fungi.

MATERIALS

The fungi that are met with in clinical practice as the common causes of the superficial and the deep mycotic infections were chosen as test organisms. These fungi and the diseases that they produce are: *Trichophyton mentagrophytes* (*gypseum*) and *Trichophyton rubrum* (*purpureum*), the common causes of Tinea pedis (athlete's foot); *Epidermophyton floccosum* (*inguinale*), the common cause of Tinea cruris (Jock strap itch); *Microsporum audouini* and *Microsporum felineum* (*lanosum*), the common causes of Tinea capitis; *Candida* (*Monilia*) *albicans*, the cause of thrush and a common cause of paronychia and vaginitis; *Blastomyces dermatitidis*, the cause of blastomycosis; *Coccidioides immitis*, the cause of coccidioidomycosis; *Sporotrichum schencki*, the cause of sporotrichosis; *Phialophora pedrosoi* (*verrucosa*), the cause of chromoblastomycosis; *Cryptococcus neoformans*, the cause of cryptococcosis; *Histoplasma capsulatum*, the cause of histoplasmosis or Darling's disease; and *Actinomyces bovis*, an anaerobic actinomycete, a cause of human actinomycosis.

The fungi of the genera *Trichophyton*, *Epidermophyton*, *Microsporum* and *Candida* were isolated by us from patients infected with these parasites. The strain of *Coccidioides immitis* was furnished to us by Dr. Charles E. Smith of the Stanford University School of Medicine. The culture of *Actinomyces bovis* was obtained from Dr. J. Howard Brown of the Johns Hopkins Medical School. Cultures of the remaining fungi were obtained from the American Type Culture Collection, Georgetown University, Washington, D. C. The strain of *Trichophyton mentagrophytes*, #640, was supplied by Dr. Chester W. Emmons, of the National Institute of Health, Bethesda, Maryland.

The 10 per cent solutions of sodium valerate, sodium caproate, sodium caprylate, sodium caprate and sodium undecylenate were supplied to us by Dr. Charles Hoffman, Director of Research of the Ward Baking Company, New York City. These solutions were prepared on the basis of the weight of the respective fatty acids and then adjusted to pH 7.4.

METHODS

I. Procedures used with Common Pathogenic Fungi

A. *The determination of fungistatic activity.* The technique employed was essentially that of Schamberg and Kolmer (10). Ten per cent solutions of sodium valerate, sodium caproate, sodium caprylate, sodium caprate and sodium undecylenate were prepared and adjusted to pH 7.4. The solutions were then added to melted dextrose agar in such amounts as to produce for each, concentrations of 0.001, 0.01, 0.1 and 1.0 per cent. The medicated agars were then adjusted to pH 7.4. Approximately 7 cc. of the medicated agar was placed in each culture tube and the tubes were then autoclaved for 15 minutes at 15 pounds of pressure and slanted. The following day the tubes were seeded with 0.5 cm. squares of freshly isolated 2 weeks old agar cultures and incubated at 30°C. Two control tubes for each fatty acid salt tested were made by seeding fungus cuttings on unmedicated dextrose agar. The cultures were observed at 24 hours intervals and the final interpretations were recorded at the end of 21 days. No attempt was made to measure partial inhibition of growth because we felt that the personal ratio of evaluation could only be eliminated by recording the concentration at which complete inhibition had occurred.

B. *The determination of fungicidal activity.* The fungous mats that had been used for seeding in the above experiments were removed from those tubes in which complete inhibition had been observed and transferred to unmedicated dextrose agar slants. The cultures were incubated at 30°C. for 21 days and then examined for growth. We were thereby able to ascertain for each fungus tested the concentration of the fatty acid salt necessary for fungicidal activity. It must be emphasized that the fungous mats had been in constant contact with the medicated dextrose agar for 21 days before being removed and it seemed essential to determine fungicidal activity for shorter exposure intervals.

Various techniques for the testing of fungicidal power (11, 12, 13, 14) were reviewed. The techniques suggested by Burlingame and Reddish (14) and by Emmons (11) were chosen.

B-1. The determination of fungicidal activity by the Burlingame and Reddish technique (14). Twenty cc. of melted dextrose agar were placed in 9 cm. petri dishes and allowed to harden. Each dextrose agar plate was inoculated with a freshly isolated culture by streaking the entire surface of the plate with a dry, cotton swab. The plates were incubated at 30°C. for 14 days. The agar cultures were then cut into 1 cm. squares and about 15 cc. of the fatty acid salt to be tested were poured over each plate so that the cultures were flooded. After periods of 5, 15, 30 and 60 minutes and 24 hours, the squares of culture and agar were removed from the plates and placed into 10 cc. of sterile dextrose broth. The excess chemical was washed from the matted cultures by shaking the broth tubes lightly for a period of 5 minutes. At the end of this time the blocks of culture and agar were removed from the broth and placed with the culture side down on to the surface of sterile dextrose agar plates. The plates were incubated at 30°C. for 3 weeks and observed for growth.

B-2. The determination of fungicidal activity by the Emmons technique (11). The principles of this test are founded upon the fact that 500,000 spores of a certain strain of *Trichophyton mentagrophytes*, so-called by Emmons #640, are killed after 15 minutes exposure to a 1–80 dilution of phenol. The degree of fungicidal activity of chemicals to be tested can then be compared with phenol and expressed in terms of phenol coefficient.

Ten plates of dextrose agar were inoculated with *Trichophyton mentagrophytes*, #640, and incubated at 30°C. for 10 days. A spore suspension in normal salt solution was then made by scraping the fungous mats with a nichrome wire needle. Care was taken in making the scrapings to avoid the removal of agar. The density of the spore suspension was determined by making a spore count. The spore count was made by employing the technique and apparatus customarily used for the enumeration of red corpuscles. The spore suspension was then adjusted so that each cubic centimeter of the suspension contained approximately 5,000,000 spores.

9.9 cc. of a 1–80 dilution of phenol were placed in each of 3 tubes and the tubes were placed in a water bath adjusted to a constant temperature of 20°C. One-tenth of a cubic centimeter of the spore suspension was added to each of the 3 tubes. At intervals of 5, 10 and 15 minutes a 4 mm. loopful of the spore-chemical suspension from these tubes was removed and placed into tubes containing 9.9 cc. of dextrose broth. The inoculated broth tubes were incubated at 30°C. and examined for growth after a few days, and at the end of 14 days. For the testing of the fatty acid salts, dilutions of 1–5, 1–10, 1–100 and 1–1000 of each of the salts were made. 9.9 cc. of each of the dilutions for each fatty acid salt were placed in tubes and inoculated with the spore suspension. The experiments were conducted in triplicate and the technique was identical to that described above for phenol.

C. *The determination of the relative toxicity of the fatty acid salts.* Solutions of the various fatty acid salts were prepared so that 1 cc. contained 1, 5, 10, 15, 25, or 50 mg., respectively. The solutions were adjusted to a pH of 7.4. Thirty to fifty-five albino mice of the Swiss or Rockefeller strain, ranging in age from 6 to 8 weeks and weighing approximately 20 grams each, were used for the testing of each fatty acid salt. The mice were divided into groups of from 5 to 10 mice to a group. Each group received daily intraperitoneal injections of a specific amount of the fatty acid salt as indicated above. The experiment was carried out for a period of 90 days.

II. Procedures used with Actinomyces bovis

Tubes of thioglycollate media containing 0.1, 1.0 and 10.0 per cent concentrations for each of the fatty acid salts, were inoculated with 0.1 cc. of the granules and media of a 4 weeks old culture of *Actinomyces bovis* and incubated at 37°C. for 4 weeks. Two control tubes were prepared for each fatty acid salt tested.

At the conclusion of these experiments the inoculum was removed as completely as possible from those tubes in which there had been obvious or complete

inhibition of growth and transferred to tubes of unmedicated thioglycollate media. These tubes with the transplanted inocula were incubated at 37°C. for 4 weeks and noted for growth. If the granules failed to increase in size or multiply during this time of incubation, we assumed that they had been killed as well as inhibited by the previous exposure to the fatty acid salts.

RESULTS

I. Experiments on Common Pathogenic Fungi

A. Fungistatic Experiments (Table I). The percentages recorded in this table for each preparation represent the concentration necessary

TABLE I

Demonstrating the Fungistatic Activity of the Sodium Salts of Some of the Fatty Acids on Common Pathogens

TYPES OF FUNGI	FATTY ACID SALTS, THEIR pH AND PERCENTAGE NECESSARY FOR COMPLETE INHIBITION OF GROWTH					
	Sodium propionate 5.5	Sodium valerate 7.4	Sodium caproate 7.4	Sodium caprylate 7.4	Sodium caprate 7.4	Sodium undecylenate 7.4
T. mentagrophytes	0.1	1.0	1.0	1.0	0.1	1.0
T. rubrum	1.0	1.0	1.0	0.1	0.1	0.1
E. floccosum	1.0	1.0	1.0	0.1	0.1	0.1
M. audouini	1.0	1.0	1.0	0.1	0.1	1.0
M. felineum	0.1	1.0	1.0	0.1	0.1	0.1
C. immitis	1.0	1.0	1.0	0.1	0.1	0.1
S. schencki	1.0	1.0	1.0	1.0	0.1	0.1
B. dermatitidis	0.01	1.0	1.0	0.1	0.1	0.1
P. pedrosoi	1.0	1.0	1.0	1.0	0.1	0.1
H. capsulatum	0.1	1.0	1.0	0.1	0.1	0.1
C. albicans	1.0	—	1.0	0.1	0.5	1.0
C. neoformans	1.0	1.0	1.0	0.1	0.1	0.1

to completely inhibit growth for a period of 3 weeks. The results for sodium propionate have been previously reported (7) and are included for the sake of comparison. For the most part, sodium caprylate, sodium caprate and sodium undecylenate were more fungistatic than the shorter chain fatty acid salts. There were exceptions. Sodium propionate inhibited the growth of *Trichophyton mentagrophytes*, *Microsporum felineum* and *Histoplasma capsulatum* equally as well as the longer chain acids, and was superior to all other preparations in its effect on Blastomyces dermatitidis.

B-1. Fungicidal Experiments (*Table II*). At the conclusion of the fungistatic experiments the fungous mats that had been used to seed the culture tubes were removed from those tubes in which there had been complete inhibition of growth. The mats were placed on slants of unmedicated dextrose agar, incubated at 30°C. and observed for growth after 21 days. We were thus able to measure the concentrations of the chemicals necessary to effect a killing action on these mats. While there was no spectacular difference in the fungicidal activity

TABLE II

Demonstrating the Fungicidal Activity of the Sodium Salts of Some of the Fatty Acids on Common Pathogens

Fungus cuttings remained on medicated dextrose agar three weeks

TYPES OF FUNGI	FATTY ACID SALTS, THEIR pH AND PERCENTAGES NECESSARY TO KILL FUNGUS CUTTING					
	Sodium propionate 5.5	Sodium valerate 7.4	Sodium caproate 7.4	Sodium caprylate 7.4	Sodium caprate 7.4	Sodium undecylenate 7.4
T. mentagrophytes	1.0	1.0	1.0	1.0	0.1	1.0
T. rubrum	1.0	1.0	1.0	0.1	0.1	0.1
E. floccosum	1.0	1.0	1.0	0.1	0.1	0.1
M. audouini	1.0	1.0	1.0	0.1	0.1	1.0
M. felineum	0.1	1.0	1.0	0.1	0.1	0.1
C. immitis	10.0	—	—	1.0	—	0.1
S. schenckii	—	5.0	—	1.0	0.1	1.0
B. dermatitidis	1.0	1.0	1.0	0.1	0.1	0.1
P. pedrosoi	1.0	1.0	1.0	1.0	1.0	1.0
H. capsulatum	1.0	1.0	1.0	0.1	0.1	0.1
C. albicans	5.0	5.0	1.0	1.0	1.0	1.0
C. neoformans	1.0	1.0	1.0	0.1	0.1	0.1

of these compounds by this technique, some general impressions were conceived. Sodium caprylate, sodium caprate, sodium undecylenate and sodium pripionate were always as effective and were usually more effective than sodium valerate and sodium caproate. Sodium propionate was usually as effective as sodium caprylate, sodium caprate and sodium undecylenate against the fungi that produce the superficial mycotic infections, but decidedly less effective against the fungi that produce the deep-seated mycotic infections. There was no difference in any of the compounds in regard to *Phialophora pedrosoi*.

B-2. (*Tables III, IV, V, VI and VII*). In an attempt to gain a

more concrete impression of the fungicidal activity of the various fatty acid salts, the Burlingame-Reddish technique was employed.

TABLE III

Fungicidal Activity of Sodium Valerate at pH 7.4

Tabulation of drug concentrations with shortest periods of exposure time necessary to kill fungi at those concentrations (Burlingame-Reddish technique)

TYPE OF FUNGUS	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME
T. mentagrophytes	0.1		1.0		10.0	30 min.
T. rubrum	0.1		1.0		10.0	30 min.
E. floccosum	0.1		1.0		10.0	
M. audouini	0.1		1.0		10.0	
M. felineum	0.1		1.0		10.0	24 hrs.
C. immitis	0.1		1.0		10.0	
S. schencki	0.1		1.0		10.0	24 hrs.
B. dermatitidis	0.1		1.0		10.0	5 min.
P. pedrosoi	0.1		1.0		10.0	
H. capsulatum	0.1		1.0		10.0	

TABLE IV

Fungicidal Activity of Sodium Caproate at pH 7.4

Tabulation of drug concentrations with shortest periods of exposure time necessary to kill fungi at those concentrations (Burlingame-Reddish technique)

TYPE OF FUNGUS	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME
T. mentagrophytes	0.1		1.0		10.0	60 min.
T. rubrum	0.1		1.0		10.0	5 min.
E. floccosum	0.1		1.0	24 hrs.	10.0	5 min.
M. audouini	0.1		1.0		10.0	5 min.
M. felineum	0.1		1.0		10.0	
C. immitis	0.1		1.0		10.0	30 min.
S. schencki	0.1		1.0		10.0	5 min.
B. dermatitidis	0.1		1.0		10.0	5 min.
P. pedrosoi	0.1		1.0		10.0	5 min.
H. capsulatum	0.1		1.0		10.0	5 min.

This method represents a fairly simple but severe test which, in a way, approaches conditions met with in clinical practice. All of the fungi,

except *Cryptococcus neoformans* and *Candida albicans*, can be tested by this technique. The growth of these 2 organisms, which is yeast-

TABLE V

Fungicidal Activity of Sodium Caprylate at pH 7.4

Tabulation of drug concentrations with shortest periods of exposure time necessary to kill fungi at those concentrations (Burlingame-Reddish technique)

TYPE OF FUNGUS	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME
T. mentagrophytes	0.1		1.0	5 min.		
T. rubrum	0.1		1.0	5 min.		
E. floccosum	0.1		1.0			
M. audouini	0.1		1.0	15 min.	10.0	5 min.
M. felineum	0.1		1.0	60 min.	10.0	5 min.
C. immitis	0.1		1.0		10.0	5 min.
S. schencki	0.1		1.0		10.0	30 min.
B. dermatitidis	0.1		1.0	15 min.	10.0	5 min.
P. pedrosoi	0.1		1.0		10.0	5 min.
H. capsulatum	0.1		1.0	24 hrs.	10.0	5 mini

TABLE VI

Fungicidal Activity of Sodium Caprate at pH 7.4

Tabulation of drug concentrations with shortest periods of exposure time necessary to kill fungi at those concentrations (Burlingame-Reddish technique)

TYPE OF FUNGUS	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME
T. mentagrophytes	0.1		1.0	24 hrs.	10.0	5 min.
T. rubrum	0.1	24 hrs.	1.0	5 min.		
E. floccosum	0.1	24 hrs.	1.0	5 min.		
M. audouini	0.1	60 min.	1.0	5 min.		
M. felineum	0.1	24 hrs.	1.0	15 min.	10.0	5 min.
C. immitis	0.1	24 hrs.	1.0	5 min.		
S. schencki	0.1	24 hrs.	1.0	24 hrs.	10.0	5 min.
B. dermatitidis	0.1	5 min.				
P. pedrosoi	0.1	24 hrs.	1.0	5 min.		
H. capsulatum	0.1	24 hrs.	1.0	5 min.		

like on dextrose agar, separates from the agar when the cultures are flooded with the testing compounds. It will be recalled too, that

after the chemical has remained in contact with the agar cultures, an attempt is made to wash the chemical free from the agar cultures so as to eliminate the possibility of the fungistatic action of testing substances. It is unlikely, however, that this can be entirely accomplished because the agar must absorb and hold a certain quantity of the chemical. Nevertheless, eliminating this criticism the test does provide a suitable method of determining the relative fungicidal activity of substances tested.

TABLE VII

Fungicidal Activity of Sodium Undecylenate at pH 7.4

Tabulation of drug concentrations with shortest periods of exposure time necessary to kill fungi at those concentrations (Burlingame-Reddish technique)

TYPE OF FUNGUS	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERCENTAGE	EXPOSURE TIME	DRUG CONCENTRATION IN PERENTAGE	EXPOSURE TIME
T. mentagrophytes	0.1	60 min.	1.0	30 min.	10.0	5 min.
T. rubrum	0.1	24 hrs.	1.0	5 min.		
E. floccosum	0.1	24 hrs.	1.0	5 min.		
M. audouini	0.1	24 hrs.	1.0	5 min.		
M. felineum	0.1		1.0	30 min.	10.0	5 min.
C. immitis	0.1	24 hrs.	1.0	30 min.	10.0	5 min.
S. schencki	0.1	24 hrs.	1.0	30 min.	10.0	5 min.
B. dermatitidis	0.1	5 min.				
P. pedrosoi	0.1		1.0	30 min.	10.0	5 min.
H. capsulatum	0.1		1.0	15 min.	10.0	5 min.

We have previously reported (7) that sodium propionate in a concentration of 20 per cent is not fungicidal. When 2 carbon atoms are added to the chain, as in the case of sodium valerate, Table III, some fungicidal activity is acquired. Slightly more fungicidal activity is demonstrated by sodium caproate, Table IV, and still more by sodium caprylate, Table V. Finally, sodium caprate, Table VI, and sodium undecylenate, Table VII, which possess 10 and 11 carbon atoms, respectively, produce the greatest degree of fungicidal activity. It, therefore, is very apparent, that as the fatty acid chain is lengthened more fungicidal activity is acquired.

B-3. (*Table VIII*). The technique for the testing of fungicidal activity as recommended by Emmons is ideal in every respect but one,

and that is it cannot be employed for all strains of pathogenic fungi. However, as this method, or a modification of this method, promises to be chosen as the standard technique for the determination of fungicidal activity, its inclusion in a study of this type is essential.

The results of this test are recorded in Table VIII and it will be observed that sodium caprate and sodium undecylenate, though not as effective as phenol, are the only fatty acids salts that we have studied extensively, which possess fungicidal activity for the spores of

TABLE VIII

Demonstrating the Fungicidal Effect of the Fatty Acid Salts for the Spores of Trichophyton Mentagrophytes, Strain #640 (Emmons)

PREPARATIONS AND HYDROGEN ION CONCENTRATION	DILUTIONS	FUNGICIDAL ACTIVITY FOR PERIOD OF EXPOSURE OF		
		5 minutes	10 minutes	15 minutes
Phenol, pH 6.0	1–80	+	+	–
Sodium propionate, pH 7.4	1–5	+	+	+
Sodium propionate, pH 5.5	1–5	+	+	+
Sodium valerate, pH 7.4	1–5	+	+	+
Sodium caproate, pH 7.4	1–5	+	+	+
Sodium heptanoate, pH 7.4	1–5	+	+	+
Sodium caprylate, pH 7.4	1–5	+	+	+
Sodium nonenoate, pH 7.4	1–5	–	–	–
Sodium caprate, pH 7.4	1–5	–	–	–
Sodium undecylenate, pH 7.4	1–5	–	–	–

+ = Growth (absence of fungicidal activity).

– = No growth (presence of fungicidal activity).

Trichophyton mentagrophytes, #640, has recently become more resistant to fungicides. A 1–70 dilution of phenol is now required to effect a killing action for an exposure of 15 minutes.

Trichophyton mentagrophytes, strain #640. In this table we have also included results using the salts of heptanoic and nonenoic acids and it appears that fungicidal activity first becomes apparent with the 9 carbon atom fatty acid.

II. Experiments with Actinomyces bovis

In Table IX are recorded the results of the fungistatic and fungicidal tests on *Actinomyces bovis*. This organism was completely inhibited and apparently killed by a 0.1 per cent concentration of sodium caprate and sodium undecylenate and by a 1.0 per cent concentration of

sodium caprylate. Actinomyces bovis was not appreciably susceptible to the shorter chain fatty acids.

TABLE IX

Demonstrating the Fungistatic and Fungicidal Effect of the Fatty Acid Salts on Actinomyces bovis

FATTY ACID SALTS AND CONCENTRATION IN PER CENT	FUNGISTATIC ACTIVITY (TUBES INCUBATED AT 37°C. FOR 28 DAYS)	FUNGICIDAL ACTIVITY (TRANSPLANTS MADE FROM TUBES AT CONCLUSION OF FUNGISTATIC EXPERIMENTS. TUBES WITH TRANSPLANTED INOCULA INCUBATED AT 37°C. FOR 28 DAYS)
Sodium valerate		
0.1	++++	++++
1.0	++++	++++
10.0	++	++++
Sodium caproate		
0.1	++++	++++
1.0	++++	++++
10.0	++	++++
Sodium caprylate		
0.1	++	++++
1.0	−	−
10.0	−	−
Sodium caprate		
0.1	−	−
1.0	−	−
10.0	−	−
Sodium undecylenate		
0.1	−	−
1.0	−	−
10.0	−	−
Controls	++++	++++

++++, +++, ++, + = Different degrees of growth.
− = No apparent growth.

III. Toxicity Experiments

The relative toxicity for the fatty acid salts was determined by administering daily various quantities of the compounds intraperitoneally to albino mice for a period of 90 days. The highest non-toxic

daily dose (Table X) for sodium propionate was 5 mg.; for sodium valerate was 25 mg.; for sodium caproate was 50 mg.; for sodium caprylate was 5 mg.; for sodium caprate was 1 mg.; and for sodium undecylenate was 1 mg. It became obvious then, that the compounds that are the most fungicidal are likewise the most toxic.

DISCUSSION

Trichophyton mentagrophytes, *Trichophyton rubrum*, *Epidermophyton floccosum*, *Microsporum audouini*, *Microsporum felineum* and *Candida albicans* are the fungi commonly responsible for the superficial mycotic infections. These parasites are frequently referred to as the "dermatophytes," and they infect the superficial layers of the skin and reside for the most part in the stratum corneum. A chemical to be used in the treatment of cutaneous mycotic infections must possess the ability to penetrate the stratum corneum and come in contact with the mycelia and arthrospores that are embedded there.

It is a matter of common knowledge that the superficial mycotic infections of the skin are likely to recur. This is particularly so for tinea pedis. There are 2 possible explanations for this. One is that reinfection takes place by contagion and the other is that the fungus lies dormant in the tissues, awaiting a period of breakdown in the host's resistance which will permit its propagation. Therefore, a chemical that is employed to cure and prevent infection must be used frequently over a long period of time.

The inflammatory reaction that accompanies the cutaneous infection is most likely a hypersensitive reaction to the infecting fungi or their products. It is quite possible, too, that some degree of temporary immunity on the part of the host is established as the result of this superficial infection. The infection, in the majority of instances, will subside and the tissues heal, with bed rest and without medication. But because the infection is not usually of a debilitating nature, an attempt is commonly made to vigorously treat the infection while the patient is ambulatory. The end result is generally over-treatment, with irritation and sensitization of the skin from the applied medicaments.

We have previously demonstrated that sodium propionate is effective in the treatment of cutaneous mycotic infections. Furthermore, we

have called attention to the fact that it can be used daily and indiscriminately over long periods of time without fear of irritation or sensitization. The clinical results from the use of sodium propionate are not dramatic and the chief criticism is that improvement manifests itself slowly. The reasons for this are apparent. Sodium propionate can inhibit the growth of fungi, but it is not a fungicide. In other words, it offers assistance to the host by inhibiting the progress of the infection while the host is mustering together its natural protective mechanisms.

It was our hope, as the result of this investigation, to find a compound that possessed all of the favorable features of sodium propionate and in addition possessed the ability to effect a more rapid recovery.

From the results of data presented in this paper, the fatty acids acquire fungicidal activity as their chain is lengthened. Of those compounds tested, sodium caprate and sodium undecylenate are the most promising fungicides. As far as the fungi that produce the superficial mycotic infections are concerned, sodium caprate and sodium undecylenate, as inhibitors of growth, are not greatly superior to sodium propionate, if sodium propionate is adjusted to pH 5.5. However, sodium caprate and sodium undecylenate are more promising fungistatic agents against the fungi that produce the deep mycotic infections.

Therefore, the longer chain fatty acid salts, that is sodium caprylate and particularly sodium caprate and sodium undecylenate are at least equal to or superior to sodium propionate and the shorter chain acids in fungistatic and fungicidal activity, regardless of the organism tested. It seemed quite possible that sodium caprate or sodium undecylenate might prove to be more effective clinically in the treatment of the cutaneous mycotic infections than sodium propionate. Such a clinical survey is now being conducted.

Therapeutic agents to be employed in the treatment of the deep mycotic infections and actinomycoses must be non-toxic because the chemical to be effective should be administered orally or parenterally. We have demonstrated that the fatty acids are relatively non-toxic for albino mice (Table X). However, sodium caprate and sodium undecylenate are more toxic than the shorter chain fatty acid salts and this fact might prevent their successful usage in the treatment of

TABLE X

The Comparative Toxic Effect of Fatty Acid Salts on Mice

COMPOUND	NO. MICE	DAILY INTRAPERITONEAL DOSE	DEATH—DAYS OF INJECTION																			SURVIVALS	
			1	2	3	4	5	6	7	8	9	10	11	13	16	18	20	24	27	28	30	60	90	No.	%
		mg.																							
Sodium propionate	5	5																						5	100
	10	5																						10	100
	5	10		2		2				1														0	0
	10	15		6	1	1		2																0	0
Sodium valerate	5	1																						5	100
	5	5																						5	100
	5	10																						5	100
	5	15																						5	100
	5	25																						5	100
	5	50	1	1										1										2	40
Sodium caproate	5	1																						5	100
	10	5																						10	100
	10	10																					4	6	60
	10	15																					2	8	80
	10	25																						10	100
	10	50																						10	100
Sodium capry-ylate	5	1																						5	100
	10	5																						10	100
	10	10		1	1	1	3	1	2				1											0	0
	10	15		1	2	6	1																	0	0
Sodium caprate	5	1										1												4	80
	10	5								1		1			1	2	1	1	1					2	20
	10	10		1	3	5	1																	0	0
	10	15		6	2		1	1																0	0
Sodium undecy-lenate	5	1																						5	100
	10	5							3											1	1	3		2	20
	10	10		4		2	4																	0	0
	10	15	1	1	1	4	2				1													0	0

the deep mycotic infections. Furthermore, it is yet to be proved that an effective blood level for these preparations in vivo can be

obtained. Therefore, until suitable animal experiments have been performed the use of fatty acids in the treatment of the deep mycotic infections and actinomycosis is inadvisable.

SUMMARY

The fungistatic and fungicidal effect in vitro of sodium valerate, sodium caproate, sodium caprylate, sodium caprate and sodium undecylenate on the common pathogens and on *Actinomyces bovis* has been studied and compared with sodium propionate.

Sodium caprylate, sodium caprate and sodium undecylenate are usually more fungistatic for the common pathogens and for *Actinomyces bovis*, than sodium propionate, sodium valerate and sodium caproate. However, sodium propionate inhibited the growth of *Trichophyton mentagrophytes*, *Microsporum felineum* and *Histoplasma capsulatum* equally as well as the longer chain fatty acid salts and was superior to all other preparations in its effect on *Blastomyces dermatitidis*.

Fungicidal activity is acquired as the fatty acid chain is lengthened. Sodium caprate and sodium undecylenate, though not strong fungicides, possess more fungicidal activity than sodium caprylate, sodium caproate and sodium valerate. Sodium propionate does not possess fungicidal activity.

The relative toxicity of the fatty acid salts for albino mice has been determined. The compounds enumerated in the order from lowest to highest degree of toxicity are: sodium caproate, sodium valerate, sodium propionate, sodium caprylate, sodium caprate and sodium undecylenate.

The in vitro effect of sodium caprate and sodium undecylenate on the fungi responsible for the cutaneous mycotic infections is sufficiently impressive to warrant the clinical trial of either preparation in the treatment of Tinea pedis, Tinea cruris, Tinea glabrosa, and Tinea capitis.

The in vitro effect of sodium caprate and sodium undecylenate on the fungi responsible for the deep mycotic infections is impressive. However, the clinical trial of these compounds in the treatment of the deep and the systemic mycotic infections and in actinomycosis should be withheld until animal experiments have been performed.

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STUDIES ON COMMON PATHOGENIC FUNGI AND ON ACTINOMYCES BOVIS

II. In Vitro Effect of Sulfonamides[1, 2]

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The sulfonamides are general cell inhibitors and their inhibitory action on bacteria is not unique. Certain viruses (1, 2), protozoa (3, 4), yeasts (5), fungi (6, 7, 8, 9) and actinomycetes (10) have been reported as inhibited by sulfonamides.

To evaluate critically the effect of sulfonamides in vitro it is of paramount importance to take into consideration that certain substances can completely or partially counteract their effect. There is sufficient data now to warrant the conclusion that interference with sulfonamide action can be due to (1) an antagonism by some specific interference with sulfonamide action, exemplified by p-aminobenzoic acid, (2) a growth stimulation on the cell unrelated to the mode of sulfonamide action, exemplified by the action of glucose and amino acids, and (3) an antagonism resulting from the formation of an inactive complex between sulfonamide and antagonist, exemplified by combinations with peptone, glucose, urea, albumin and arginine.

The inhibitory effect of some of the sulfonamides in vitro on *Trichophyton gypseum* (6, 7), *Trichophyton purpureum* (6), *Monilia albicans* (7), *Blastomyces dermatitidis* (8), *Sporotrichum schencki* (9) and *Actinomyces hominis* (10) has been reported but only in one instance (6) have the sulfonamide-antagonists been taken into account. It is the purpose of this paper to report upon the effectiveness in vitro of those sulfonamide drugs in common use, on all of the common pathogenic fungi and a strain of Actinomyces bovis, taking into account the action of the sulfonamide-antagonists.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

[2] The results of this investigation were presented in part at the meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey, May 8, 1944.

MATERIALS

The sodium salts of sulfathiazole, sulfadiazine and sulfamerazine were employed because they are more soluble in media than their parent substances. Dextrose broth and blood dextrose, serum dextrose and sucrose nitrate agars were used as media for the fungus experiments and thioglycollate media was employed for the experiments with Actinomyces bovis. Sodium sulfadiazine and sodium sulfamerazine begin to crystallize out in dextrose broth and thioglycollate media at a concentration of 500 mg. per cent. Sodium sulfathiazole crystallizes out slightly in a concentration of 1000 mg. per cent in dextrose broth but is completely soluble in thioglycollate media in a concentration of 1000 mg. per cent.

The fungi that are met with in clinical practice as the causes of the superficial and the deep mycotic infections were chosen as test organisms. The fungi and the diseases they produce are: *Trichophyton mentagrophytes* (*gypseum*) and *Trichophyton rubrum* (*purpureum*), the common causes of Tinea pedis; *Epidermophyton floccosum* (*inguinale*), the common cause of Tinea cruris; *Microsporum audouini* and *Microsporum felineum* (*lanosum*), the common causes of Tinea capitis; *Candida* (*Monilia*) *albicans*, the cause of thrush and a common cause of vaginitis and chronic paronychia; *Blastomyces dermatitidis*, the cause of blastomycosis; *Coccidioides immitis*, the cause of coccidioidomycosis; *Sporotrichum schencki*, the cause of sporotrichosis; *Phialophora pedrosoi* (*verrucosa*), the cause of chromoblastomycosis; *Cryptococcus neoformans*, the cause of cryptococcosis; *Histoplasma capsulatum*, the cause of histoplasmosis or Darling's disease; and Actinomyces bovis, an anaerobic actinomycete, a cause of human actinomycosis.

The fungi of the genera *Trichophyton*, *Epidermophyton*, *Microsporum* and *Candida* were isolated by us from patients infected with these parasites. The strain of *Coccidioides immitis* was furnished to us by Dr. Charles E. Smith of the Stanford University School of Medicine. Cultures of the remaining fungi were obtained from the American Type Culture Collection, Georgetown University, Washington, D. C. The strain of *Trichophyton mentagrophytes*, #640, was obtained from Dr. Chester W. Emmons, of the National Institute of Health, Bethesda, Maryland.

METHODS

Determination of Sulfonamide-antagonists. The quantity of each of the sulfonamides necessary to completely neutralize their respective antagonists present in the dextrose broth and the thioglycollate media was determined. Sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine were added to tubes of dextrose broth and thioglycollate media in such amounts as to produce final concentrations for each sulfonamide of 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg. per cent. The tubes were inoculated with a 4 mm. loopful of a 24 hours broth culture of staphylococcus aureus diluted 1 to 10. In 24 hours the tubes were examined for turbidity. The lowest concentration of each sulfonamide tested which would just permit apparent growth was taken as the amount of that sulfonamide necessary to completely neutralize its respective sulfonamide-antagonists present in the media.

Fungistatic Experiments. Tubes of dextrose broth containing 50, 100, 500, and 1000 mg. per cent concentrations each of sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine, plus the excess amount of respective sulfonamide required to neutralize sulfonamide-antagonists, were inoculated with the fungi. In the instance of *Candida albicans*, of *Cryptococcus neoformans*, and of *Sporotrichum schencki*, the inoculum was a 4 mm. loopful of a 2 weeks old broth culture which contained for the most part spores. In the case of the other organisms, 0.5 cm. squares of agar cultures, which contained spores and mycelia, were used as inocula. Two control tubes were made for each fungus tested. The tubes were incubated at 30°C. and examined after 21 days for growth.

The fungistatic activity of sodium sulfathiazole was then determined by employing the technique of Schamberg and Kolmer (11). This sulfonamide was chosen in preference to sodium sulfadiazine and sodium sulfamerazine simply because it alone would not crystallize out in the agar in a concentration of 1000 mg. per cent.

Three types of media were used for this experiment; dextrose blood agar,[3] dextrose serum agar[4] and sucrose nitrate agar.[5] The first 2 media obviously contain sulfonamide-antagonists. Sucrose nitrate agar is essentially free from antagonists. Sodium sulfathiazole was added to each of the different media so that a final concentration of 1000 mg. per cent was obtained. No attempt was made to add an excess of sodium sulfathiazole for the neutralization of antagonists. The agar plates were inoculated with fungus mats cut from freshly isolated fungous

[3] Dextrose blood agar. Nutrient agar 100 cc.; whole blood 5–10 cc.; dextrose (20%) 5 cc.

[4] Dextrose serum agar. Nutrient agar 100 cc.; serum 5–10 cc.; dextrose (20%) 5 cc.

[5] Sucrose nitrate agar. Agar 15 gm.; magnesium sulphate 0.5 gm.; potassium chloride 0.5 gm.; di-potassium phosphate 1.0 gm.; ferric sulphate 0.01 gm.; sodium nitrate 2.0 gm.; sucrose 30.0 gm. (This formula in grams per liter.)

cultures. Unmedicated agar plates were inoculated and were used as controls. The plates were incubated at 30°C. for 21 days, and observed for growth.

Fungicidal Experiments. At the conclusion of the fungistatic tests, the fungous mats were removed from the dextrose broth culture tubes in which there had been complete inhibition of growth and transferred to tubes of unmedicated dextrose broth, incubated at 30°C. and observed for growth after a period of 21 days. We were thus able to determine the concentration of the sulfonamides necessary to effect a killing action on the fungous mats.

By employing the technique of Emmons (12) it is possible to determine the fungicidal activity of the sulfonamides for a certain strain of *Trichophyton mentagrophytes* without the necessity of considering the various sulfonamide-antagonists. The principles of this test are founded upon the fact that 500,000 spores of a certain strain of *Trichophyton mentagrophytes*, so called by Emmons, #640, are killed after 15 minutes of exposure to a 1–80 dilution of phenol. The degrees of fungicidal activity of chemicals to be tested can then be compared with phenol and expressed in terms of phenol coefficient.

Ten plates of dextrose agar were inoculated with *Trichophyton mentagrophytes*, strain #640, and allowed to grow at 30°C. for 10 days. A spore suspension in normal salt solution was made by scraping the fungus mats from the dextrose agar plates with a nichrome wire needle. Care was taken in making the scrapings to avoid the removal of agar. The density of the spore suspension was determined by making a spore count. The spore count was made by employing the technique and apparatus customarily used for the enumeration of red corpuscles. The spore suspension was then adjusted so that each cubic centimeter of the suspension contained approximately 5,000,000 spores.

9.9 cc. of a 1–80 dilution of phenol were placed in 3 tubes and the tubes were placed in a water bath adjusted to a constant temperature of 20°C. One-tenth of a cubic centimeter of the spore suspension was then added to each of the 3 tubes. At intervals of 5, 10 and 15 minutes a 4 mm. loopful of the spore-chemical suspension from these tubes was removed and placed in tubes containing 9.9 cc. of dextrose broth. The inoculated broth tubes were incubated at 30°C. and examined for growth after a few days and at the end of 14 days. For the testing of the sulfonamides a 25 per cent solution of sodium sulfathiazole, a 25 per cent solution of sodium sulfadiazine and a 5 per cent solution of sodium sulfamerazine were employed. 9.9 cc. of each of these solutions were placed in tubes and inoculated with the spore suspension. The experiments were conducted in triplicate and the technique was identical to that described for phenol.

Experiments with Actinomyces bovis: Tubes of thioglycollate media containing 50, 100, 500 and 1000 mg. per cent concentrations each of sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine were inoculated with a 4 weeks old culture of *Actinomyces bovis*. A control tube was prepared for each sulfonamide. The quantity of each of the sulfonamides necessary to neutralize their respective antagonists present in this media was not added, because we had found that this excess of sulfonamide produced so much crystallization in the thioglycollate media that it was difficult to interpret our results.

The tubes were incubated at 37°C. for 4 weeks and then noted for growth. In those tubes in which there had been complete inhibition of growth, the original inoculum was for the most part removed and transferred to tubes of unmedicated thioglycollate media and incubated at 37°C. for 4 weeks. At the end of this time, if the granules had not increased in size or multiplied, we assumed that they had been killed.

TABLE I

Bacteriostatic Effect of Sodium Sulfathiazole, Sodium Sulfadiazine and Sodium Sulfamerzine in Dextrose Broth and Thioglycollate Media on a 24 Hours Broth Culture of Staphylococcus aureus, Diluted 1 to 10

SULFONAMIDE AND CONCENTRATION IN MEDIA	DEXTROSE BROTH	THIOGLYCOLLATE MEDIA
Sodium Sulfathiazole		
80 mg. %	+++	++++
90 mg. %	++	++++
100 mg. %	+	+++
200 mg. %	−−	+++
300 mg. %	−−	++
400 mg. %	−−	+
500 mg. %	−−	−−
Sodium Sulfadiazine		
100 mg. %	++++	++++
200 mg. %	++++	++++
300 mg. %	++++	++++
400 mg. %	++++	+++
500 mg. %	++++	++
600 mg. %	+++	+
700 mg. %	++	−−
800 mg. %	+	−−
900 mg. %	−−	−−
1000 mg. %	−−	−−
Sodium Sulfamerazine		
100 mg. %	+++	+++
200 mg. %	+++	++
300 mg. %	++	++
400 mg. %	+	+
500 mg. %	−−	−−

++++, +++, ++, + = Different degrees of turbidity. −− = No apparent growth.

RESULTS

I. Experiments with Common Pathogenic Fungi

In Table I are recorded the minimal bacteriostatic concentrations of each of the sulfonamides tested for a 1 to 10 dilution of a 24 hours

broth culture of staphylococcus aureus, in dextrose broth and in thioglycollate media. This minimal bacteriostatic concentration for each sulfonamide was taken by us as the concentration of that sulfonamide necessary to counteract antagonists present in the media. This concentration varied for each sulfonamide and for each media tested. The minimal bacteriostatic concentrations for sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine in dextrose broth were 100, 800 and 400 mg. per cent respectively; and in thioglycollate media were 400, 600 and 400 mg. per cent respectively. We are aware that these figures would vary somewhat depending upon the organism tested and upon the size of the inocula.

A. Fungistatic Experiments. In Table II are recorded the results of the experiments to determine the fungistatic activity of the sodium salts of sulfathiazole, sulfadiazine and sulfamerazine. The quantity of each sulfonamide necessary to counteract its respective antagonists present in the media was added in excess to the concentrations used for testing. The concentrations used for testing were 50, 100, 500 and 1000 mg. per cent solutions of the sulfonamides. Actually, however, when the excess sulfonamide was added, the tubes contained 150, 200, 600 and 1100 mg. per cent concentrations of sodium sulfathiazole; 850, 900, 1300 and 1800 mg. per cent concentrations of sodium sulfadiazine; and 450, 500, 900 and 1400 mg. per cent concentrations of sodium sulfamerazine.

For the most part the sulfonamides possessed little or no significant fungistatic activity. However, *Microsporum audouini* was completely inhibited by a 500 mg. per cent concentration of sodium sulfathiazole; *Cryptococcus neoformans* was completely inhibited by a 1000 mg. per cent concentration of sodium sulfadiazine and sodium sulfamerazine; *Phialophora pedrosoi* was completely inhibited by a 50 mg. per cent concentration of sodium sulfamerazine; and *Histoplasma capsulatum* was completely inhibited by a 50 mg. per cent concentration of sodium sulfathiazole.

In Table III are recorded the results of fungistatic experiments with a 1000 mg. per cent concentration of sodium sulfathiazole which had been incorporated into 3 different agar preparations. Sucrose nitrate agar does not contain any substances that specifically interfere with the action of sulfonamides. The whole blood and serum dextrose

TABLE II

Demonstrating the Fungistatic Activity of the Sodium Salts of Sulfathiazole, Sulfadiazine and Sulfamerazine on Common Pathogens

TYPES OF FUNGI	GROWTH OF CONTROLS	SODIUM SULFATHIAZOLE				SODIUM SULFADIAZINE				SODIUM SULFAMERAZINE			
		Mg. per cent											
		50	100	500	1000	50	100	500	1000	50	100	500	1000
T. mentagrophytes	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
T. rubrum	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+	+ + +	+ + +	+ + +	+ +
E. floccosum	+ + +	+ + +	+ + +	+ + +	+	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
M. audouini	+	+	+	−	−	+	+	+	−	+	+	+	−
M. felineum	+ + +	+ + +	+ + +	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
C. neoformans	+ + +	+ + +	+ + +	+ +	+	+ + +	+ +	+	−	+ + +	+ +	+	+
C. albicans	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
B. dermatitidis	+ + +	+ + +	+ + +	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
C. immitis	+ + +	+ + +	+ + +	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
S. schencki	+ + +	+ + +	+ +	+ +	+	+ + +	+ + +	+ +	+	+ + +	+ +	+ +	+
P. pedrosoi	+ +	+	−	−	−	+	+	−	−	−	−	−	−
H. capsulatum	+	−	−	−	−	+	−	−	−	+	−	−	−

agars on the other hand contain sulfonamide-antagonists. This experiment was conducted for the purpose of observing the differences in sulfonamide action in different media and also for the purpose of comparing the results with those obtained by employing dextrose broth.

It is apparent by comparing the degree of growth in the control plates for the 3 different media that sucrose nitrate agar provides a poorer nutritional environment for the organisms than the other 2 agars. This, of course, is important in studying the effectiveness of

TABLE III

Demonstrating the Variation of Fungistatic Activity of a 1000 Mg. Per Cent Concentration of Sodium Sulfathiazole on Common Pathogens in Regard to Media Employed

TYPES OF FUNGI	BLOOD DEXTROSE AGAR WITH SOD. SULFATHIAZOLE	BLOOD DEXTROSE AGAR	SERUM DEXTROSE AGAR WITH SOD. SULFATHIAZOLE	SERUM DEXTROSE AGAR	SUCROSE NITRATE AGAR WITH SOD. SULFATHIAZOLE	SUCROSE NITRATE AGAR
T. mentagrophytes	++	+++	++	+++	+	+
T. rubrum	++	++	+	++	+	+
E. floccosum	++	+++	++	+++	+	+
M. aldouini	+	++	+	++	−	+
M. felineum	++	+++	+++	+++	+	+
C. neoformans	++	++	+	+++	−	+
C. albicans	+++	+++	++	+++	+	+
B. dermatitidis	++	+++	++	+++	−	+
S. schenckii	++	+++	+	+++	+	++
P. pedrosoi	−	+	−	++	−	++
H. capsulatum	+	++	−	++	+	++

sulfonamides in vitro because it is well known that sulfonamide action is greater if growth-stimulating substances are minimal.

Microsporum audouini, *Phialophora pedrosoi*, *Cryptococcus neoformans* and *Blastomyces dermatitidis* were completely inhibited by a 1000 mg. per cent solution of sodium sulfathiazole. Therefore, the results obtained by employing the sucrose nitrate agar are very similar to those obtained by employing dextrose broth to which had been added excess sulfonamide to counteract the antagonists.

However, when the sodium sulfathiazole was incorporated in the whole blood and serum dextrose agar, its inhibiting effects were appreciably reduced. *Histoplasma capsulatum* and *Phialophora pedro-*

soi were completely inhibited in the serum dextrose agar and Phialophora pedrosoi was completely inhibited in the whole blood dextrose agar. It would seem, therefore, from these experiments alone that of all the pathogenic fungi, *Phialophora pedrosoi* and *Histoplasma capsulatum* are the most susceptible to the inhibiting effect of the sulfonamides. This, in turn, was corroborated and better demonstrated in the previous fungistatic experiments (Table II).

B. Fungicidal Experiments. At the completion of those fungistatic experiments which were conducted in dextrose broth, the fungous mats were removed from the tubes in which there had been complete inhibition and transferred to tubes of unmedicated dextrose broth,

TABLE IV

Demonstrating the Fungicidal Activity of the Sodium Salts of Sulfathiazole, Sulfadiazine and Sulfamerazine on those Common Pathogens Whose Growth Was Completely Inhibited by These Sulfonamides

Fungus cuttings remained in medicated dextrose broth 3 weeks

TYPES OF FUNGI	SULFONAMIDES AND THEIR PERCENTAGES NECESSARY TO KILL FUNGI CUTTINGS		
	Sodium sulfathiazole	Sodium sulfadiazine	Sodium sulfamerazine
	mg. %	*mg.* %	*mg.* %
M. audouini	—	1000	1000
P. pedrosoi	500	500	500
H. capsulatum	100	—	1000

incubated at 30°C. for 21 days and then noted for growth. We were thus able to determine the concentrations of each sulfonamide which effected a killing action on the fungous mats as well as an inhibition of growth. In Table IV are recorded the concentrations of sulfonamides which effected a killing action on the fungi. The sulfonamides for the most part are poor fungicidal agents, however, *Microsporum audouini* was killed by a 1000 mg. per cent concentration of sodium sulfadiazine and sodium sulfamerazine; *Phialophora pedrosoi* was killed by a 500 mg. per cent concentration of sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine; and *Histoplasma capsulatum* was killed by a 100 mg. per cent concentration of sodium sulfathiazole and a 1000 mg. per cent concentration of sodium sulfadiazine.

In Table V are recorded the results of the fungicidal activity of the sulfonamides tested against a spore suspension of *Trichophyton mentagrophytes*, strain #640. This particular organism is a rather resistant species of *Trichophyton mentagrophytes* against which Emmons compares the fungicidal activity of compounds to phenol. This technique is ideal in every respect but one and that is it can not be used for all genera of pathogenic fungi. However, the technique or a modification of the technique, promises to be chosen as a standard method of determining fungicidal activity and for this reason alone its inclusion in a study of this type is essential. Sodium sulfathiazole

TABLE V

Demonstrating the Lack of Fungicidal Activity of the Sodium Salts of Sulfathiazole, Sulfadiazine and Sulfamerazine for the Spores of Trichophyton Mentagrophytes Strain #640 (Emmons)

COMPOUNDS TESTED	DILUTION	FUNGICIDAL ACTIVITY FOR PERIODS OF EXPOSURE OF		
		5 minutes	10 minutes	15 minutes
Phenol	1–80	+	+	−
Sod. sulfathiazole	1–4	+	+	+
Sod. sulfadiazine	1–4	+	+	+
Sod. sulfamerazine	1–20	+	+	+

− = no growth (presence of fungicidal activity).

+ = growth (absence of funicidal activity).

Trichophyton mentagrophytes, #640, has recently become more resistant to fungicides. A 1–70 dilution of phenol is now required to effect a killing action for an exposure of 15 minutes.

and sodium sulfadiazine in concentrations of 25 per cent and sodium sulfamerazine in a concentration of 5 per cent are not fungicidal. It was impossible to prepare a more concentrated, yet satisfactory, solution of sodium sulfamerazine.

II. Experiments with Actinomyces bovis

In Table VI are recorded the results of the fungistatic activity of the sodium salts of sulfathiazole, sulfadiazine and sulfamerazine for *Actinomyces bovis*. The experiments were conducted in thioglycollate media. The quantities of each of the sulfonamides necessary to counteract the specific antagonists present in the media (Table I)

were not added in excess to the concentrations used for testing. Previously we had noted that by adding this additional amount of sulfonamide to the media so much crystallization occurred that it became impossible to interpret our results. From the data recorded in Table VI, it will be observed that the sulfonamides inhibit the

TABLE VI

Demonstrating the Fungistatic and Fungicidal Activity of the Sodium Salts of Sulfathiazole, Sulfadiazine and Sulfamerazine for Actinomyces bovis

SULFONAMIDE AND CONCENTRATION IN MEDIA	FUNGISTATIC ACTIVITY (TUBES INCUBATED AT 37°C. FOR 28 DAYS)	FUNGICIDAL ACTIVITY (TRANSPLANTS MADE FROM TUBES AT CONCULUSION OF FUNGISTATIC EXPERIMENTS. TUBES WITH TRANSPLANTED INOCULA INCUBATED AT 37°C. FOR 28 DAYS)
Sodium sulfathiazole		
50 mg. %	++	++++
100 mg. %	++	++++
500 mg. %	–	–
1000 mg. %	–	–
Sodium sulfadiazine		
50 mg. %	+++	++++
100 mg. %	+++	++++
500 mg. %	–	–
1000 mg. %	–	–
Sodium sulfamerazine		
50 mg. %	+++	++++
100 mg. %	+++	++++
500 mg. %	–	–
1000 mg. %	–	–
Controls	++++	++++

++++, +++, ++ = Different degrees of growth.
– = No apparent growth.

growth of *Actinomyces bovis*. Furthermore, it is likely that greater inhibiting action actually exists since considerable amounts of sulfonamide-antagonists are present in the thioglycollate media (Table I).

Also in Table VI are recorded the results of the fungicidal activity of the sulfonamides for Actinomyces bovis. It is apparent that the concentration of the sulfonamides necessary to completely inhibit

the growth of *Actinomyces bovis* also can effect a killing action on this organism.

DISCUSSION

From these data it is evident that the sodium salts of sulfathiazole, sulfadiazine and sulfamerazine are not sufficiently strong inhibitors of fungi in vitro to warrant their general use clinically in the treatment of the superficial and the deep mycotic infections.

The fungi included in the genera of *Trichophyton*, *Epidermophyton*, *Microsporum* and *Candida*, commonly referred to as "the dermatophytes," are the causes of the superficial mycotic infections. These parasites infect the superficial layers of the skin and reside for the most part in the stratum corneum. Of these genera of fungi there are only 1 or 2 species in each genera that are of clinical importance. *Trichophyton mentagrophytes* and *Trichophyton rubrum* are the common causes of Tinea pedis (athlete's foot); *Epidermophyton floccosum* is the common cause of Tinea cruris (Jock strap itch); *Microsporum audouini* and *Microsporum felineum* are the common causes of Tinea capitis; and *Candida albicans* is the cause of thrush and a common cause of paronychia and vaginitis.

Lewis and Hopper (7) studied the in vitro effect of sulfanilamide, sodium sulfapyridine, sulfathiazole, sodium sulfathiazole, sulfadiazine and sodium sulfadiazine on *Trichophyton mentagrophytes* and *Candida albicans*. They demonstrated that a 1 per cent solution of sulfanilamide completely inhibited Trichophyton mentagrophytes and that the other sulfonamides exhibited slight fungistatic activity for this fungus. *Candida albicans* was not inhibited by any of the sulfonamides tested. No comment was made in their report that they had considered the possible role of sulfonamide-antagonists in their media.

Dimond and Thompson (6) in their report on the effect of sulfanilamide, sulfapyridine, sulfathiazole, sulfaquanidine and sodium sulfadiazine on *Trichophyton mentagrophytes* and *Trichophyton rubrum*, did take into consideration the possible role of sulfonamide-antagonists in their media. They demonstrated that a 0.1 per cent solution of sulfanilamide was fungistatic for both parasites and that the other sulfonamides had no inhibiting effect on growth in concentrations as high as 0.5 per cent.

To our knowledge there is no previous report in the literature concerning the effect of the sulfonamides in vitro on *Epidermophyton floccosum*, *Microsporum audouini* and *Microsporum felineum*.

From the results summarized in Table II it is apparent that sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine fail to significantly inhibit the growth of "the dermatophytes." Microsporum audouini was completely inhibited by a 500 mg. per cent concentration of sodium sulfathiazole and by a 1000 mg. per cent concentration of sodium sulfadiazine and sodium sulfamerazine, but we doubt that the inhibitory effect in vitro is significant enough to warrant the use of these sulfonamides in the treatment of Tinea capitis due to this organism.

The fungi that produce the deep and the systemic mycotic infections are *Blastomyces dermatitidis*, *Sporotrichum schencki*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Phialophora pedrosi*, *Histoplasma capsulatum* and also, occasionally, *Candida albicans*.

Noojin and Callaway (8) tested the in vitro efficacy of sulfanilamide, sulfapyridine, sodium sulfapyridine, sulfathiazole, sodium sulfathiazole, sulfadiazine and sulfaquanidine against *Blastomyces dermatitidis*. They concluded that all of these compounds would probably be ineffective in the treatment of blastomycosis if employed orally or parenterally but that sulfadiazine, sulfanilamide, sulfapyridine and sodium sulfapyridine, in the order enumerated, might be effective if applied locally in the form of a powder or solution. As a verification of their prophecy, these authors are soon to publish a case report on a patient with cutaneous blastomycosis that was successfully treated by the local application of a 25 per cent solution of sodium sulfapyridine (9).

Noojin and Callaway (9) have recently reported a similar study on the effect of sulfonamides in vitro against *Sporotrichum schencki* and it is to be observed from their results that the sulfonamides fail to inhibit this parasite in such a degree as to warrant their use orally or parenterally in the treatment of sporotrichosis, but the authors recommend the local application of either sulfanilamide or sodium sulfapyridine.

From the results of our in vitro tests with sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine against *Blastomyces dermatitidis* and *Sporotrichum schenki* and with the knowledge of the antagonists to sulfonamide action present in tissue fluids and purulent

exudate, we are more conservative in recommending these sulfonamides even for local application. At any rate one is not justified in recommending their clinical use in the treatment of systemic blastomycosis and sporotrichosis.

We have also demonstrated that *Coccidioides immitis* and *Cryptococcus neoformans* are not inhibited sufficiently in vitro to warrant the clinical use of sulfonamides in the treatment of coccidioidomycosis and cryptococcosis. However, Phialophora pedrosoi was completely inhibited by a 50 mg. per cent concentration of sodium sulfamerazine and *Histoplasma capsulatum* was completely inhibited by a 50 mg. per cent concentration of sodium sulfathiazole and sodium sulfadiazine. Furthermore, we have demonstrated that a 500 mg. per cent concentration of each of the 3 sulfonamides tested are capable of killing *Phialophora pedrosoi* if the fungus is exposed to the drugs for a period of 21 days. *Histoplasma capsulatum* was killed in a similar manner by a 100 mg. per cent concentration of sodium sulfathiazole. Therefore, the possible clinical effectiveness of sodium sulfamerazine in the treatment of chromoblastomycosis and sodium sulfathiazole in the treatment of histoplasmosis might be anticipated.

Human actinomycosis is usually caused by an anaerobic actinomycete, referred to commonly as *Actinomyces bovis*. A few cases, however, are caused by an aerobic actinomycete properly classified now as *Nocardia asteroides*. Although Actinomyces bovis is commonly anaerobic, certain strains will grow aerobically, especially after frequent transfers have been made.

Cutting and Gebhardt (10) have reported upon the effectiveness of sulfanilamide, sulfathiazole and sulfadiazine in vitro against *Actinomyces hominis* From their report in which they describe the inhibitory effectiveness of the sulfonamides on this organism in aerobic and anaerobic conditions, it seems quite likely that they were concerned with a strain of *Actinomyces bovis* which was capable of growing under both aerobic and anaerobic conditions. They concluded that sulfathiazole and sulfadiazine were more effective than sulfanilamide and were capable of completely inhibiting growth in concentrations of 50 mg. per cent.

We were unable to confirm entirely the results of Cutting and Gebhardt (10). It is true that we did obtain partial inhibition of growth with sodium sulfathiazole in a concentration of 50 mg. per cent,

but we did not obtain complete inhibition until a concentration of 500 mg. per cent was obtained. Complete inhibition was also noted for both sulfadiazine and sodium sulfamerazine at 500 mg. per cent concentration. It is true that a 50 mg. and 100 mg. per cent concentration of both sodium sulfadiazine and sodium sulfamerazine produced some slight inhibition but the difference between these tubes and the controls was so small that we are inclined to rely on complete inhibition for the proper evaluation of results since the personal equation in interpretation is eliminated. However, it is quite possible that the inhibiting effect of the sulfonamides is greater than our results would suggest because we were unable to discount the effect of sulfonamide-antagonists present in the media. It is to be noted that Cutting and Gebhardt (10) made no mention of the sulfonamide-antagonists present in their media.

There are numerous clinical notes in the literature proclaiming the effectiveness of sulfanilamide (13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23), sulfapyridine (19, 24, 25), succinyl sulfathiazole (26) and sulfadiazine (23, 27) in the treatment of human actinomycosis due to *Actinomyces bovis*. Hall and Spink (28) reported a failure with sulfamerazine and Benbow, Smith and Grismon (29) have reported 2 cases of human actinomycosis due to *Nocardia asteroides* treated successfully with sulfanilamide and sulfadiazine. In the majority of these reported cases surgical measures, iodide and x-ray therapy supported the sulfonamide therapy and it is certainly impossible to ascribe the entire clinical improvement to the action of the sulfonamides.

It is our impression, which is founded on the results of data presented in this paper and the critical analysis of clinical reports in the literature, that sulfonamide therapy alone in the treatment of human actinomycosis is likely to be met with failure. However, if sulfonamide therapy is supported by necessary surgical intervention, indicated x-ray therapy and the proper administration of iodides, the chances to effect a cure for this disease are made optimal.

SUMMARY

The fungistatic and fungicidal effect of sodium sulfathiazole, sodium sulfadiazine and sodium sulfamerazine, in vitro, on the common pathogenic fungi and *Actinomyces bovis* has been studied.

The inhibiting effect of these sulfonamides on the growth of the fungi that produce the superficial mycotic infections, that is, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum*, *Microsporum audouini*, *Microsporum felineum* and *Candida albicans*, is not sufficiently impressive to warrant their use in the treatment of the skin infections that they produce.

The inhibiting effect of these sulfonamides on the growth of the fungi that produce the deep and the systemic mycotic infections, that is, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Sporotrichum schencki*, *Coccidioides immitis*, *Phialophora pedrosoi*, and *Histoplasma capsulatum*, is only impressive for *Phialophora pedrosoi* and *Histoplasma capsulatum*, the respective causes of chromoblastomycosis and histoplasmosis. The clinical trial of sodium sulfamerazine in the treatment of chromoblastomycosis and sodium sulfathiazole in the treatment of histoplasmosis is warranted.

The inhibiting effect of these sulfonamides on the growth of *Actinomyces bovis* is not spectacular and conservatism should be displayed in regard to expectancy of cure from their use in the treatment of human actinomycosis.

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STUDIES ON COMMON PATHOGENIC FUNGI AND ON ACTINOMYCES BOVIS

III. In Vitro Effect of Penicillin[1, 2]

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If one can assume that the intensity of interest in regard to the antibacterial effect of penicillin is now at its zenith, then the nadir for this exists in regard to its effect on fungi. Brief statements have been made by Abraham et al (1) and by Fisher (2) that *Actinomyces bovis* is susceptible to penicillin, in vitro. Hobby et al (3) have reported that Cryptococcus hominis is susceptible but that *Monilia albicans* is not susceptible to penicillin, in vitro. These few isolated reports are the extent of the in vitro studies on penicillin for pathogenic fungi and for *Actinomyces bovis*.

It is the purpose of this paper to report upon the effect of penicillin, in vitro, on all of the common pathogenic fungi and on *Actinomyces bovis*.

MATERIALS AND METHODS

The fungi that are met with in clinical practice as the common causes of the superficial and the deep mycotic infections were chosen as test organisms. These fungi and the diseases that they produce are: *Trichophyton mentagrophytes* (*gypseum*) and *Trichophyton rubrum* (*purpureum*), the common causes of Tinea pedis (athlete's foot); *Epidermophyton floccosum* (*inguinale*), the common cause of Tinea cruris (Jock strap itch); *Microsporum audouini* and *Mircosporum felineum* (*lanosum*), the common causes of Tinea capitis; *Candida* (*Monilia*) *albicans*, the cause of thrush and a common cause of paronychia and vaginitis; *Blastomyces dermatitidis*, the cause of blastomycosis; *Coccidioides immitis*, the cause of coccidioidomycosis; *Sporotrichum schencki*, the cause of sporotrichosis; *Phialophora pedrosoi* (*verrucosa*), the cause of chromoblastomycosis; *Cryptococcus neoformans*, the cause of cryptococcosis; *Histoplasma capsulatum*, the cause of histoplasmosis

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

[2] The results of this investigation were presented in part at the meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey, May 8, 1944.

or Darling's disease; and *Actinomyces bovis*, an anaerobic actinomycete, a cause of human actinomycosis.

The fungi of the genera *Trichophyton*, *Epidermophyton*, *Microsporum* and *Candida* were isolated by us from patients infected with these parasites. The strain of *Coccidioidis immitis* was furnished to us by Dr. Charles E. Smith of the Stanford University School of Medicine. Cultures of the remaining fungi were obtained from the American Type Culture Collection, Georgetown University, Washington, D. C. The strain of *Trichophyton mentagrophytes*, #640, was supplied by Dr. Chester W. Emmons, of the National Institute of Health, Bethesda, Maryland.

Fungistatic Experiments with the Common Pathogens. Purified penicillin was employed entirely in the experiments and fresh solutions were prepared in each instance when it was added or re-added to media. Tubes of dextrose broth containing 0.1, 0.5, 1.0 and 10.0 Oxford units respectively of penicillin per cubic centimeter of media were inoculated with the fungi. In the cases of *Candida albicans*, *Cryptococcus neoformans* and *Sporotrichum schencki*, the inoculum was a 4 mm. loopful of a 2 weeks old culture which contained for the most part spores. In the instance of the other organisms, 0.5 cm. squares of freshly isolated 2 weeks old agar cultures, which contained spores and mycelia, were used as inocula. Two control tubes were made for each test organism. The tubes were incubated at 30°C. for 14 days and observed for growth. Because penicillin is not stable, and because the fungi grow slowly, fresh penicillin was added every 48 hours to the tubes so that the original concentrations of penicillin, i.e., 0.1, 0.5, 1.0 and 10.0 Oxford units per cubic centimeter of media could be maintained throughout the period of 14 days.

Fungicidal Experiments with Trichophyton mentagrophytes, #640. By employing the technique of Emmons (4) it is possible to determine the fungicidal activity of penicillin for *Trichophyton mentagrophytes*. The principles of this test are founded upon the fact that 500,000 spores of a certain strain of *Trichophyton mentagrophytes*, so called by Emmons, #640, are killed after 15 minutes of exposure to a 1–80 dilution of phenol. The degree of fungicidal activity of chemicals to be tested can then be compared with phenol and expressed in terms of phenol coefficient.

Ten plates of dextrose agar were inoculated with *Trichophyton mentagrophytes*, #640, and allowed to grow at 30°C. for 10 days. A spore suspension in normal salt solution was made by scraping the fungous mats from the dextrose agar plates with a nichrome wire needle. Care was taken in making the scrapings to avoid the removal of agar. The density of the spore suspension was determined by making a spore count. The spore count was made by employing the technique and apparatus customarily used for the enumeration of red corpuscles. The spore suspension was then adjusted so that each cubic centimeter of the suspension contained approximately 5,000,000 spores.

9.9 cc. of a 1–80 dilution of phenol were placed in each of 3 tubes and the tubes were placed in a water bath adjusted to a constant temperature of 20°C. One-

tenth of a cubic centimeter of the spore suspension was then added to each of the three tubes. At the intervals of 5, 10 and 15 minutes a 4 mm. loopful of the spore-chemical suspension from these tubes was removed and placed in tubes containing 9.9 cc. of dextrose broth. The inoculated broth tubes were incubated at 30°C. and examined for growth after a few days and at the end of 14 days. Identical experiments were performed with penicillin in a concentration of 500 Oxford units per cubic centimeter of diluent.

Experiments with Actinomyces bovis. Tubes of thioglycollate media containing 0.005, 0.01, 0.05, 0.1, 0.5, 1.0 and 10.0 Oxford units of penicillin, respectively, were inoculated with 0.1 cc. of the granules and media of a 4 weeks old culture of

TABLE I

Demonstrating the Results of Fungistatic Experiments with Penicillin on Common Pathogenic Fungi

TYPES OF FUNGI	GROWTH OF CONTROLS	OXFORD UNITS OF PENICILLIN PER CC. OF MEDIA			
		0.1	0.5	1.0	10.0
T. mentagrophytes	++++	++++	++++	++++	++++
T. rubrum	++++	++++	++++	++++	++++
E. floccosum	+++	+++	+++	+++	+++
M. audouini	+	++++	+++	+++	+++
M. felineum	++++	++++	++++	++++	++++
C. neoformans	++++	++++	+++	+++	+++
C. albicans	++++	++++	++++	++++	++++
B. dermatitidis	+++	++++	++++	++++	+++
C. immitis	+++	++++	++++	++++	+++
S. schenckii	++++	++++	++++	++++	++++
P. pedrosoi	+++	++++	++++	+++	+++
H. capsulatum	+	+	+	+	+

+, ++, +++, ++++ = Different degrees of growth at the end of 14 days.

Actinomyces bovis, and incubated at 37°C. for 3 weeks. Freshly prepared penicillin was re-added to the tubes every 48 hours so that the original concentrations of penicillin could be maintained throughout the entire period of 3 weeks. The growth in the penicillin treated cultures was compared with the growth in 2 control cultures.

At the conclusion of these experiments the inocula was removed as completely as possible from those tubes in which there had been obvious or complete inhibition of growth and transferred to tubes of unmedicated thioglycollate media. These tubes with the transplanted inocula were incubated at 37°C. for 4 weeks and noted for growth. If the granules failed to increase in size or multiply during this time of incubation, we assumed that they had been killed as well as inhibited by the previous exposure to penicillin.

RESULTS

It is to be observed from the results recorded in Table I, that penicillin, even in a concentration of 10.0 Oxford units per cubic

TABLE II

Demonstrating the Lack of Fungicidal Activity of Penicillin for the Spores of Trichophyton mentagrophytes, Strain #640 (Emmons)

COMPOUNDS TESTED	DILUTION	FUNGICIDAL ACTIVITY FOR PERIODS OF EXPOSURE OF		
		5 minutes	10 minutes	15 minutes
Phenol	1–80	+	+	−
Penicillin	500 Oxford units per cc.	+	+	+

− = No growth (presence of fungicidal activity).
+ = Growth (absence of fungicidal activity).

Trichophyton mentagrophytes, #640, has recently become more resistant to fungicides. A 1–70 dilution of phenol is now required to effect a killing action for an exposure of 15 minutes.

TABLE III

Demonstrating the Fungistatic and Fungicidal Activity of Penicillin for Actinomyces bovis

PENICILLIN (OXFORD UNITS PER CC. OF MEDIA)	FUNGISTATIC ACTIVITY (TUBES INCUBATED AT 37°C. FOR 28 DAYS)	FUNGICIDAL ACTIVITY (TRANSPLANTS MADE FROM TUBES AT CONCLUSION OF FUNGISTATIC EXPERIMENTS. TUBES WITH TRANSPLANTED INOCULA INCUBATED AT 37 C. FOR 28 DAYS)
0.005	+++	++++
0.01	−	−
0.05	−	−
0.1	−	−
0.5	−	−
1.0	−	−
10.0	−	−
Controls	++++	++++

++++ = Degree of growth.
− = No apparent growth.

centimeter of media, does not inhibit the growth of any of the pathogenic fungi. As a matter of fact, in many instances penicillin seemed to stimulate the growth of the fungi. Furthermore in Table II it is

to be noted that 500 Oxford units of penicillin per cubic centimeter of diluent are incapable of killing the spores of *Trichophyton mentagrophytes*, #640.

Actinomyces bovis, on the other hand was inhibited and apparently killed by a concentration of 0.01 Oxford unit of penicillin per cubic centimeter of media (Table III). *Actinomyces bovis*, in regard to its susceptibility to penicillin, is more like a bacterium than a fungus. This fact is also borne out in in vitro experiments with the sulfonamides (5).

DISCUSSION

Fungi included in the genera, *Trichophyton*, *Epidermophyton*, *Microsporum* and *Candida* are the common causes of the superficial mycotic infections. The mycelia and the arthrospores of these fungi reside for the most part in the superficial layers of the skin. These infections are treated most effectively therefore by the local application of medicaments at the site of infection. The Floreys (6) have demonstrated that penicillin applied locally is effective in the treatment of certain bacterial cutaneous infections. However, they have shown that the applications must be made at frequent intervals and that a concentration of from 250 to 500 Oxford units of penicillin per cubic centimeter of vehicle is essential. This extravagant use of penicillin might be warranted in the treatment of cutaneous fungous infections if the parasites responsible for such infections were susceptible to penicillin. However, penicillin does not inhibit the growth of these fungi in vitro. If anything, penicillin seems to stimulate their growth.

Recently we have seen, in consultation with Dr. J. E. Moore, 3 patients whose superficial mycotic infections were either reinactivated or exacerbated by penicillin which had been administered intramuscularly for the treatment of infections due to Treponema pallidum. This represents an interesting in vivo correlation to our in vitro observations.

The fungi that produce the deep and the systemic mycotic infections are *Blastomyces dermatitidis*, *Sporotrichum schencki*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Phialophora pedrosoi*, *Histoplasma capsulatum* and also, occasionally, *Candida albicans*.

We were unable to confirm the observations of Hobby et al (3)

that *Cryptococcus neoformans* (*hominis*) is susceptible to penicillin, in vitro. Furthermore we were unable to demonstrate that any of the fungi responsible for the deep mycotic infections were susceptible to penicillin. As in the case of the fungi responsible for the superficial mycotic infections, penicillin seemed to stimulate their growth. Because of these in vitro observations and because of the flare up of dermatophytosis which was coincident to penicillin therapy, penicillin in the treatment of the deep mycotic infections is contraindicated.

Although Actinomyces bovis is inhibited and apparently killed in vitro by concentrations of penicillin which could be conceivably attained in vivo, the clinical use of penicillin in the treatment of human actinomycosis has not been accompanied by spectacular results. Keefer et al (7) reported improvement in 1 patient and failure in 2 patients with human actinomycosis. Herrell (8) reported satisfactory results in 2 patients and unsatisfactory results in 1 patient with maxillofacial actinomycosis. The Floreys (6) reported failure in 2 patients with actinomycosis but concluded that the results were not informative because the dosage had been inadequate. They also reported a "possibly effective result" in a patient with a streptothrix infection. They stated that the streptothrix infection was probably caused by an actinomycete. Lyons (9) reported that 4 patients with actinomycosis were temporarily improved after penicillin therapy but that the patients had not been followed for a long enough period.

It must be concluded that the reported results on the use of penicillin in the treatment of human actinomycosis are no more impressive than the results reported for other therapeutic measures and that as in the case of the sulfonamides (5) necessary surgical intervention, adequate x-ray therapy and appropriate iodide medication are essential adjuncts if optimal results are to be obtained in the treatment of this chronic infection.

SUMMARY

The fungistatic and fungicidal effect of purified penicillin, in vitro, on the common pathogens and on *Actinomyces bovis* has been studied.

Purified penicillin in a concentration of 10 Oxford units per cubic centimeter of culture media failed to inhibit the growth of *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floc-*

cosum, *Microsporum audouini*, *Microsporum felineum*, *Candida albicans*, *Blastomyces dermatitidis*, *Sporotrichum schencki*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Phialophora pedrosoi*, and *Histoplasma capsulatum*.

Penicillin, in many instances, stimulated the growth of pathogenic fungi, in vitro.

The use of penicillin in the treatment of the superficial and the deep mycotic infections is unwarranted and contraindicated.

Penicillin in a concentration of 0.01 Oxford unit per cubic centimeter of culture media inhibits and apparently kills *Actinomyces bovis*.

Clinical reports on the use of penicillin in the treatment of human actinomycosis have not been sufficiently impressive to warrant unrestricted enthusiasm on the part of the clinician for this mode of therapy.

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PROPIONATE AND UNDECYLENATE OINTMENTS IN THE TREATMENT OF TINEA PEDIS AND AN IN VITRO COMPARISON OF THEIR FUNGISTATIC AND ANTIBACTERIAL EFFECTS WITH OTHER OINTMENTS[1, 2, 3, 4, 5]

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The clinical problems associated with cutaneous mycotic infections during peaceful times are usually of an insignificant nature. But the problems that arise concomitant to such infections during a time of military combat in tropical areas are momentous and it is not until such an emergency arises that physicians become aware of the inadequacies of erstwhile therapeutic measures with which they have been perfectly satisfied.

Tincture of iodine, Castellani's paint, and preparations containing salicylic and benzoic acids are effective agents in the treatment of cutaneous mycotic infections, but they can neither be used carelessly nor indiscriminately. There is a need for a preparation that can pro-

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

[2] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the U. S. Navy. The opinions and views set forth in this article are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

[3] We wish to express our appreciation to Dr. Lowell J. Reed and to Dr. Margaret Merrell, Department of Biostatistics, Johns Hopkins University, for their advice on the compilation of data and for their assistance in the appraisal of our results.

[4] We are greatly indebted to the authorities of the United States Naval Academy. Their interest and complete cooperation made this study possible.

[5] The results of this investigation were presented in part at the meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey, May 8, 1944.

[6] Commander, Medical Corps, United States Naval Reserve.

vide superior, or at least equally effective results without entailing the risk of tissue damage due to irritation or hypersensitization.

The fungi included in the genera of *Trichophyton*, *Epidermophyton*, *Microsporum* and *Candida*, commonly referred to as "the dermatophytes", are the causes of the superficial mycotic infections. Only 1 or 2 species of each genera are of clinical importance. *Trichophyton mentagrophytes* (*gypseum*) and *Trichophyton rubrum* (*purpureum*) are the common causes of Tinea pedis (athlete's foot); *Epidermophyton floccosum* (*inguinale*) is the common cause of Tinea cruris (Jock strap itch); *Microsporum audouini* and *Microsporum felineum* (*lanosum*) are the common causes of Tinea capitis; and *Candida* (*Monilia*) *albicans* is the cause of thrush and a common cause of vaginitis and paronychia. These fungi infect the superficial layers of the skin and the mycelia and arthrospores reside for the most part in the stratum corneum. The inflammatory reaction that accompanies the infection is a manifestation of hypersensitivity to the fungi or their products. Some immunity is developed as a result of an infection, but this is short-lived. It is common for the superficial mycotic infections to recur and this is particularly so for Tinea pedis. There are 2 possible explanations for this. One is that reinfection takes place by contagion from an outside source and the other is that the fungus lies dormant in the tissues, awaiting a period of breakdown in the host's resistance which will permit its propagation.

A compound, to be effective in the treatment of superficial mycotic infections, must first possess the power to inhibit the growth of fungi or actually kill the fungi. Secondly, it must be able to penetrate the stratum corneum and come into contact with the mycelia or arthrospores that are imbedded there. Penetration can be abetted by incorporating the compound into vehicles that possess the power of penetration in their own right. Thirdly, the compound should possess an antibacterial effect, because fungous infections are quite frequently complicated by secondary bacterial infection and the hypersensitive reaction to this bacterial infection. Fourthly, the compound and the vehicle in which it is placed should be neither irritating nor sensitizing.

We have previously reported that sodium propionate was effective as a fungistatic agent and was also effective in the treatment of cu-

taneous mycotic infections (1, 2). More recently we have studied the in vitro effect of sodium valerate, sodium caproate, sodium caprylate, sodium caprate and sodium undecylenate on the common pathogenic fungi (3). As a result of the latter studies we concluded that sodium caprate and sodium undecylenate not only possessed fungistatic activity comparable or superior to sodium propionate, but also possessed fungicidal activity which sodium propionate lacked.

Although the clinical results that we reported for sodium propionate were promising, there was one unavoidable and important criticism. That is, clinical improvement was slow. We had employed a 10 per cent concentration of sodium propionate in a petrolatum base. It seemed plausible to us that if we should increase the concentration of sodium propionate and incorporate it in a water soluble base to enhance penetration, we might be able to improve our results.

It is the purpose of this paper to report upon the clinical effectiveness of propionate-propionic acid ointment prepared in a "carbowax" base and undecylenate-undecylenic acid ointment prepared in a "carbowax" base in the treatment of tinea pedis. We shall also compare the fungistatic and antibacterial effects of these 2 ointments in vitro with one-half and full strength Whitfield's ointments, 10 per cent ammoniated mercury ointment, 5 per cent sulfathiazole ointment and 0.5 per cent tyrothricin ointment.

METHODS AND MATERIALS

I. In Vitro Experiments

The agar cup-plate technique described by Ruehle and Brewer (4) was employed to determine the fungistatic and antibacterial effects of the various ointments.

Dr. Chester W. Emmons of the National Institute of Health, Bethesda, Maryland, supplied us with *Trichophyton mentagrophytes*, strain #640. This strain is employed by Emmons in determining the relative fungistatic and fungicidal properties of compounds, and was used by us as a test organism in the fungistatic experiments.

Staphylococcus aureus and beta hemolytic streptococcus are common secondary bacterial contaminants of cutaneous mycotic infections. A coagulase positive strain of staphylococcus aureus and a Group A type of beta hemolytic streptococcus, furnished to us by Dr. Eleanor Bliss, were employed as test organisms for determining the antibacterial effect of the various ointments.

A suspension of the spores of *Trichophyton mentagrophytes*, # 640, was prepared. Ten plates of dextrose agar were inoculated with *Trichophyton mentagrophytes*, # 640, and allowed to grow at 30°C. for 10 days. A spore suspension in normal salt solution was made by scraping the fungous mats from the dextrose agar plates with a nichrome wire needle. Care was taken in making the scrapings to avoid the removal of agar. The material was filtered through cotton to remove mycelial filaments and then the density of the spore suspension was determined by making a spore count. The spore count was made by employing the technique and apparatus customarily used for the enumeration of red corpuscles. The spore suspension was then adjusted so that each cubic centimeter of the suspension contained approximately 5,000,000 spores.

Dextrose agar was melted and allowed to cool to 40°C. To each 15 cc. of agar were added 5,000,000 spores of *Trichophyton mentagrophytes*, # 640. The inoculated agar was then poured into a sterile petri dish, in the center of which had been placed a sterile glass cylinder. The cylinder measured 1 cm. in diameter and 1 cm. in height. After the inoculated agar had hardened the cylinder was removed. This left a cup in the center of the agar plate. The bottom of the cup was then sealed with 0.05 cc. of the inoculated agar and the ointment to be tested was inserted in the evacuated space above. Porous clay tops were used on all petri dishes to prevent the condensation of moisture. The plates were incubated at 30°C. for 96 hours and then read for inhibition. If a zone of inhibition occurred about the cup, the diameter of the zone was measured and recorded.

The methods and materials for determining the antibacterial effects of the ointments were essentially the same as those described for the fungistatic experiments. There were some exceptions. In the experiments with staphylococcus aureus, 0.1 cc. of a 24 hour broth culture was used to inoculate 15 cc. of blood agar base (Baltimore Biological Laboratory). The plates were incubated at 37° for 48 hours and then examined for inhibition. In the experiments with beta hemolytic streptococcus, 0.4 cc. of a 24 hour blood broth culture was used to inoculate 15 cc. of blood agar base which had been enriched with 5% whole rabbit blood. The plates were incubated at 37°C. for 24 hours and then noted for inhibition.

The fungistatic and antibacterial effects of propionate-propionic acid ointment, 5 and 10 per cent undecylenate-undecylenic acid ointments, one-half and full strength Whitfield's ointments, 10 per cent ammoniated mercury ointment, 5 per cent sulfathiazole ointment and 0.5 per cent tyrothricin ointment were determined. To enable a just comparison of their respective effectiveness, the ointments were prepared in similar bases. We were aware that the degree of penetration in the agar would depend a great deal upon the type of ointment base employed.

The propionate-propionic acid ointment and the 10 per cent undecylenate-undecylenic acid ointment were prepared and supplied to us by the Mycoloid Laboratories, Little Falls, New Jersey. The formulae are:

Propionate-propionic acid ointment	
Sodium propionate	16.4
Propionic acid	3.6
Propylene glycol	5.0
N-propyl alcohol	10.0
Zinc stearate	5.0
"Carbowax (4000)"	35.0
Water sufficient to make	100.0

Undecylenate-undecylenic acid ointment 10 per cent	
Undecylenic acid	10.0
Triethanolamine	6.0
Propylene glycol	14.0
"Carbowax (1500)"	10.0
"Carbowax (4000)"	40.0
Water sufficient to make	100.0

The 5 per cent undecylenate-undecylenic acid ointment was prepared and supplied to us by Wallace and Tiernan Products, Belleville, New Jersey. The formula is:

Undecylenic acid	5.0
Triethanolamine	3.0
Zinc peroxide	10.0
*"Methocel," 15 cps	3.0
Propylene glycol	35.0
Zinc stearate	8.0
"Carbowax (1500)"	36.0

The one-half and full strength Whitfield's ointments, the 10 per cent ammoniated mercury ointment, the 5 per cent sulfathiazole ointment and the 0.5 per cent tyrothricin ointment were prepared by Robert S. Fuqua, Head Pharmacist of the Johns Hopkins Hospital.

The base for each of these ointments was the same and was prepared as follows:

Cetyl alcohol, pure	12.0	Melt on hot water or steam bath.
†Stearyl alcohol	4.5	
Oxycholesterol absorption base	8.0	
Cottonseed oil	10.0	
Glyceryl monoricinoleate	3.0	
40% solution of "carbowax (1500)" in hot distilled water	60.0	Dissolve aerosol paste in "carbowax" solution.
Aerosol-OT (10% aqueous)	2.5	

The aqueous solution was added to the fatty mixture and stirred rapidly while being cooled in first a tepid and then a cold water bath.

The "carbowaxes" are difficult to combine into homogenous ointment bases containing both fatty substances and water unless alcohols and glycols are also present in the mixtures. The aim in this experiment was to evaluate the fungistatic and antibacterial activity of salicylic and benzoic acids, ammoniated mer-

* Methyl cellulose.

† "Stenol" (E. I. duPont de Nemours Company).

cury, sulfathiazole and tyrothricin. We therefore wished to eliminate entirely from the ointment base substances that might possess a fungistatic or antibacterial effect in their own right.

II. In Vivo Experiments

One hundred and twenty midshipmen at the United States Naval Academy with clinical evidence of Tinea pedis and with laboratory confirmation of fungous infection were divided into 3 groups. One group of 39 men was treated with propionate-propionic acid ointment,[7] another group of 40 men was treated with undecylenate-undecylenic acid ointment,[8] and a control group of 41 was treated with ointment base alone. The men were instructed to apply the ointment under the toes, over the toes, between the toes and over the soles of the feet every evening. The ointment was to be removed every morning with soap and water or with a towel. No other form of medication was issued. All the men were re-examined 1, 3, 5, and 6 weeks after the institution of therapy. At these times, careful clinical notes were made in regard to the presence of intertrigo, maceration, fissuring, scaling, denudation and dyshidrosis. No attempt was made to classify them as "improved", "worse" or the "same", or in any way make comparisons with the condition of the feet on previous examinations. The feet were pronounced clear only if they were in perfect condition. Any slight degree of scaling was classified as "scaling". It is quite likely that scaling is not always a manifestation of fungous infection and it is for this reason that we have tabulated our data by including and by omitting scaling.

Scrapings were made at the time of each examination for direct examination for fungus material and for cultures.

At the conclusion of the experiment the data were assembled and submitted to Dr. Lowell J. Reed, Department of Biostatistics, Johns Hopkins University, for appraisal.

RESULTS

I. In Vitro Experiments

In Table I are recorded and in Figures I, II and III are illustrated the results of the fungistatic and antibacterial effects, in vitro, of the various ointments. This is a dramatic and revealing demonstration of the superior efficacy of the propionate-propionic acid ointment not only for its fungistatic effect, but also for its antibacterial effect.

[7] Propionate-propionic acid ointment: sodium propionate 16.4; propionic acid 3.6; propylene glycol 5.0; n-propyl alcohol 10.0; "carbowax (4000)" 35.0; zinc stearate 5.0; water sufficient to make 100.0.

[8] Undecylenate-undecylenic acid ointment: undecylenic acid 10.0; triethanolamine 6.0; propylene glycol 14.0; "carbowax (1500)" 10.0; "carbowax (4000)" 40.0; water sufficient to make 100.0.

In its inhibitory effect on *Trichophyton mentagrophytes*, #640, which is an unusually resistant strain of *Trichophyton mentagrophytes*, the propionate-propionic acid ointment is only paralleled by the 10 per cent undecylenate-undecylenic acid ointment. Full strength Whitfield's ointment is significantly surpassed. This fact does not necessarily mean that the propionate-propionic acid ointment or the undecylenate-undecylenic acid ointment will be far superior to Whitfield's ointment in the treatment of cutaneous mycotic infections. The keratolytic, instead of the fungistatic, effect of Whitfield's ointment must be greatly responsible for the benefit that is derived from this type of medication. In regard to Whitfield's ointment, it is interesting to observe that the fungistatic effect of one-half strength Whitfield's ointment is essentially the same as full strength Whitfield's ointment. There is little difference, also, between 10 per cent ammoniated mercury and Whitfield's ointment.

TABLE I

Demonstrating the Fungistatic and Antibacterial Effects of Various Ointments, in Vitro, by the Agar Cup-Plate Technique

TYPES OF OINTMENTS	pH	ZONE OF INHIBITION FOR		
		T. mentagrophytes after 96 hrs.	Staphylococcus aureus after 48 hrs.	B. hem. strep. after 24 hrs.
		cm.	cm.	cm.
Propionate-propionic acid	5.5	6.5	2.5	2.8
Undecylenate-undecylenic acid 10 per cent	5.5	6.0	1.6	2.4
Undecylenate-undecylenic acid 5 per cent	6.5	5.3	1.8	2.2
Whitfield's—full strength	3.5	3.7	1.4	2.3
Whitfield's—one-half strength	4.0	3.5	1.2	1.9
Ammoniated mercury 10 per cent	5.5	3.3	2.2	1.9
Sulfathiazole 5 per cent	5.5	2.8 (partial)	3.0 (partial)	2.1
Tyrothricin 0.5 per cent	6.0			1.0

The remarkable antibacterial effect of the propionate-propionic acid ointment was not anticipated. The fact that one preparation is superiorly effective against both fungi and bacteria is extraordinary indeed. The antibacterial effect of propionate-propionic acid ointment against staphylococcus aureus is only approximated by the 10 per cent ammoniated mercury and the 5 per cent sulfathiazole oint-

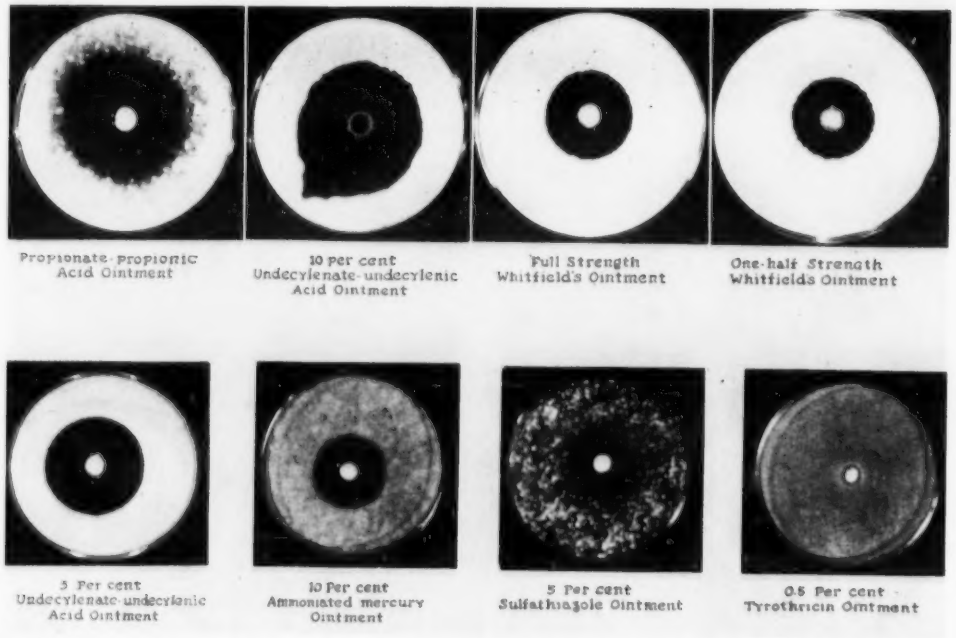
Propionate-propionic Acid Ointment
10 per cent Undecylenate-undecylenic Acid Ointment
Full Strength Whitfield's Ointment
One-half Strength Whitfields Ointment
5 Per cent Undecylenate-undecylenic Acid Ointment
10 Per cent Ammoniated mercury Ointment
5 Per cent Sulfathiazole Ointment
0.5 Per cent Tyrothricin Ointment

FIG. 1. ILLUSTRATING THE FUNGISTATIC EFFECTS OF VARIOUS OINTMENTS ON TRICHOPHYTON MENTAGROPHYTES, ×640

(Agar cup-plate technique of Ruehle and Brewer.) Clear areas about cups represent zones of inhibition

(Agar cup-plate technique of Ruehle and Brewer.) Clear areas about cups represent zones of inhibition

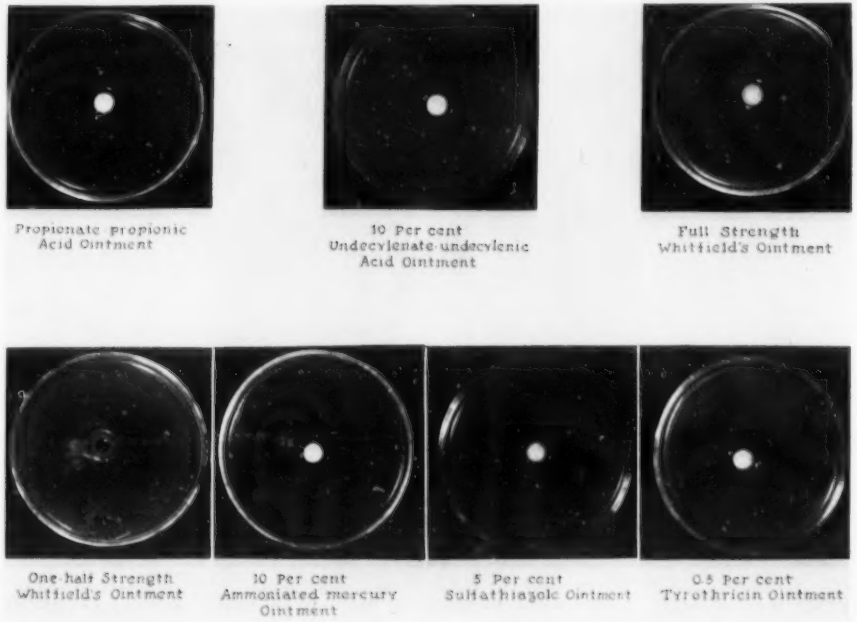
Propionate-propionic Acid Ointment
10 Per cent Undecylenate-undecylenic Acid Ointment
Full Strength Whitfield's Ointment
One-half Strength Whitfield's Ointment
10 Per cent Ammoniated mercury Ointment
5 Per cent Sulfathiazole Ointment
0.5 Per cent Tyrothricin Ointment

FIG. 2. ILLUSTRATING THE ANTIBACTERIAL EFFECTS OF VARIOUS OINTMENTS ON STAPHYLOCOCCUS AUREUS

(Agar cup-plate technique of Ruehle and Brewer.) Clear areas about cups represent zones of inhibition

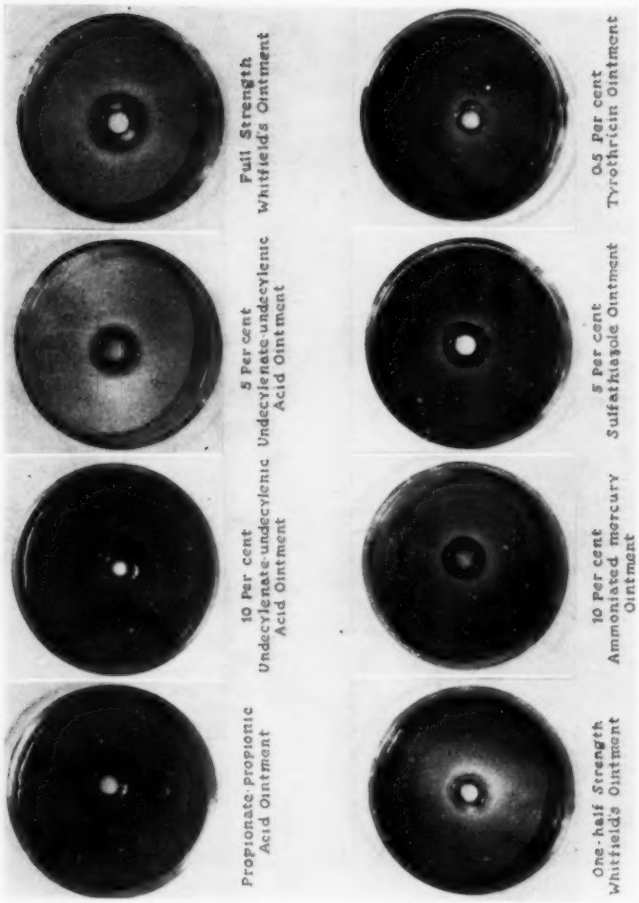
Propionate-propionic Acid Ointment
10 Per cent Undecylenate-undecylenic Acid Ointment
5 Per cent Undecylenate-undecylenic Acid Ointment
Full Strength Whitfield's Ointment
One-half Strength Whitfield's Ointment
10 Per cent Ammoniated mercury Ointment
5 Per cent Sulfathiazole Ointment
0.5 Per cent Tyrothricin Ointment

FIG. 3. ILLUSTRATING THE ANTIBACTERIAL EFFECTS OF VARIOUS OINTMENTS ON BETA HEMOLYTIC STREPTOCOCCUS

(Agar cup-plate technique of Ruehle and Brewer.) Clear areas about cups represent zones of inhibition

ments, and its effect against the beta hemolytic streptococcus is imitated only by the undecylenate-undecylenic acid ointments, full strength Whitfield's ointment, and 5 per cent sulfathiazole ointment. But regardless of the testing organism, whether it was *Trichophyton mentagrophytes*, #640, staphylococcus aureus or beta hemolytic streptococcus, the propionate-propionic acid ointment excelled all other preparations in ability to inhibit growth.

The possibility then arose that the remarkable activity possessed by the propionate-propionic acid ointment might be abetted by any

TABLE II

Demonstrating the Fungistatic and Antibacterial Effects of Propionate-Propionic Acid Ointment, in Vitro, after Deleting Individual Ingredients

INGREDIENT DELETED	pH	ZONE OF INHIBITION FOR T. mentagrophytes	INHIBITORY EFFECT LOST	ZONE OF INHIBITION FOR Staphylococcus aureus	INHIBITORY EFFECT LOST
		cm.	*per cent*	*cm.*	*per cent*
Propionic acid	8.0	2.6	60	1.9	24
Sodium propionate	5.5	5.0	23	1.8	28
Propionic acid and Sodium propionate	8.0		100		100
Zinc stearate	6.5	4.6	29	2.4	4
N-propyl alcohol	5.5	6.5	0	2.3	8
Control—nothing deleted	5.5	6.5		2.5	

one or a combination of ingredients present in the formula, and that its inhibitory powers were not confined entirely to the action of the sodium propionate and propionic acid. The results tabulated in Table II and illustrated in Figure IV clearly demonstrate that the significant fungistatic and antibacterial action of the ointment is due entirely to the combination of sodium propionate and propionic acid. When these 2 ingredients are deleted the ointment loses all of its fungistatic and antibacterial activity Furthermore the experiments demonstrate the remarkable effectiveness of the propionic acid for when this is withdrawn the ointment loses 60 per cent of its fungistatic and 24 per cent of its antibacterial effect. When zinc

(Agar cup-plate technique of Ruehle and Brewer.) Clear areas about cups represent zones of inhibition

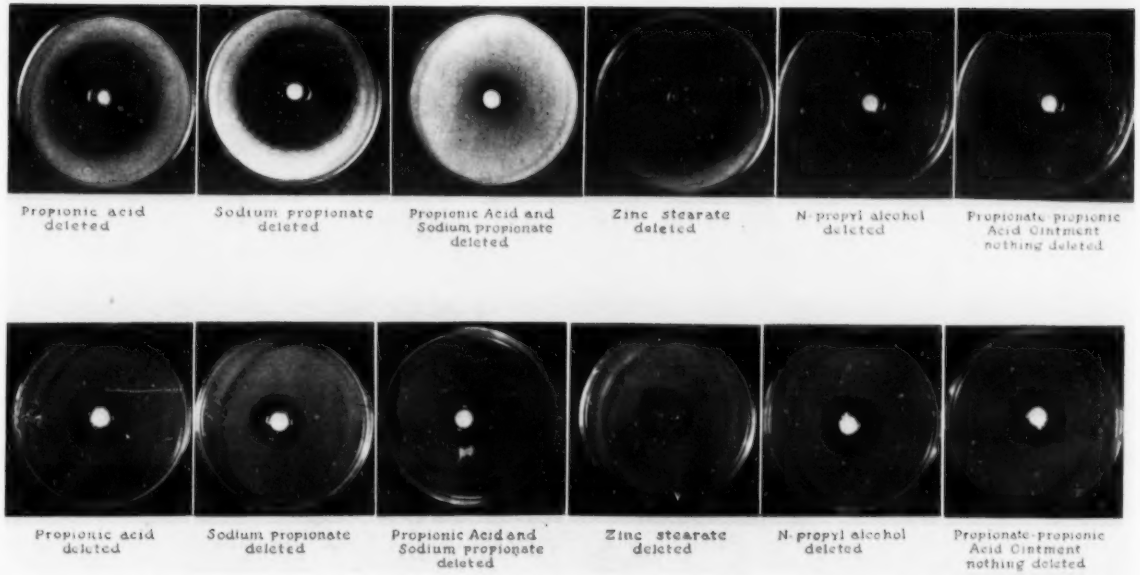

FIG. 4. ILLUSTRATING THE FUNGISTATIC EFFECTS ON TRICHOPHYTON MENTAGROPHYTES, #640 (UPPER ROW) AND ANTIBACTERIAL EFFECTS ON STAPHYLOCOCCUS AUREUS (LOWER ROW) OF PROPIONATE-PROPIONIC ACID OINTMENT AFTER DELETING INDIVIDUAL INGREDIENTS

(Agar cup-plate technique of Ruehle and Brewer.) Clear areas about cups represent zones of inhibition

stearate, which was added to stiffen the ointment base, was removed 29 per cent of the fungistatic but only 4 per cent of the antibacterial activity of the ointment was lost. The removal of the sodium propionate did not severely effect the action of the ointment in vitro. However, it seems quite probable that when sodium propionate is applied to the skin it soon breaks down to form propionic acid. Hence the intrinsic value of the sodium propionate in the formula.

Similar experiments were performed by deleting ingredients in the undecylenate-undecylenic acid ointment. It is clearly demonstrated

TABLE III

Demonstrating the Fungistatic and Antibacterial Effects of Undecylenate-Undecylenic Acid Ointment, in Vitro, after Deleting Individual Ingredients

INGREDIENT DELETED	pH	ZONE OF INHIBITION FOR T. mentagrophytes	INHIBITORY EFFECT LOST	ZONE OF INHIBITION FOR Staphylococcus aureus	INHIBITORY EFFECT LOST
		cm.	*per cent*	*cm.*	*per cent*
Undecylenic acid	8.0		100		100
Propylene glycol	5.5	6.0	0	1.6	0
Triethanolamine	5.0	6.0	0	1.5	6
Control—nothing deleted	5.5	6.0		1.6	

by the results in Table III and by the illustrations in Figure V, that the entire fungistatic and antibacterial effects of this ointment are due to the presence of undecylenic acid.

II. In Vivo Experiments

In Table IV are recorded the comparative effects of propionate-propionic acid ointment and undecylenate-undecylenic acid ointment on the microscopic presence of fungous material in scrapings. At the onset of treatment there was no significant difference between the 3 groups in regard to the percentage of positive slides. This indicated that the groups were comparable. The percentage positive at this time was between 85 and 93.

After 1 week of treatment there was a comparable fall for both treated groups in the percentages of positive slides.

(Agar cup-plate technique of Ruehle and Brewer.) Clear areas about cups represent zones of inhibition

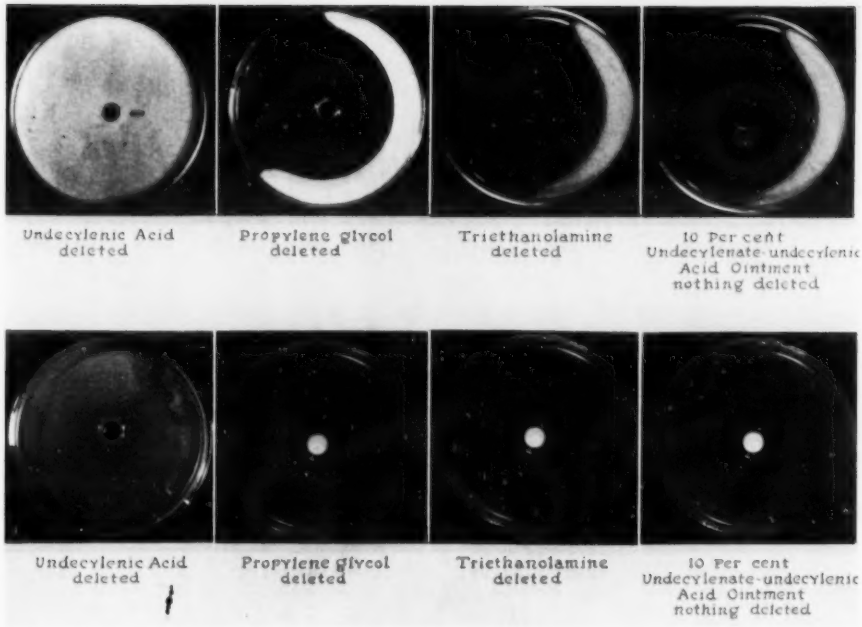
Undecylenic Acid deleted
Propylene glycol deleted
Triethanolamine deleted
10 Per cent Undecylenate-undecylenic Acid Ointment nothing deleted
Undecylenic Acid deleted
Propylene glycol deleted
Triethanolamine deleted
10 Per cent Undecylenate-undecylenic Acid Ointment nothing deleted

FIG. 5. ILLUSTRATING THE FUNGISTATIC EFFECTS ON TRICHOPHYTON MENTAGROPHYTES, #640 (UPPER ROW) AND ANTIBACTERIAL EFFECTS ON STAPHYLOCOCCUS AUREUS (LOWER ROW) OF 10 PER CENT UNDECYLENATE-UNDECYLENIC ACID OINTMENT AFTER DELETING INDIVIDUAL INGREDIENTS.

(Agar cup-plate technique of Ruehle and Brewer.) Clear areas about cups represent zones of inhibition

After 3 weeks of treatment all 3 groups showed a lower percentage positive than at the onset of treatment, between 20 and 48 per cent being positive at this time. The differences between the 2 treated groups were insignificant, but these groups did show significantly lower percentages positive than the control group.

After 5 weeks of treatment the men became careless in following instructions and there was evidence of this in the results. They were then encouraged to adhere to treatment instructions and the effects of this were observed 1 week later, that is, after 6 weeks of treatment.

TABLE IV

Comparing the Effects of Propionate-Propionic Acid Ointment and Undecylenate-Undecylenic Acid Ointment on the Presence of Fungous Material in Scrapings Made at the Site of Infection

TREATMENT GROUP	ONSET			TIME AFTER ONSET OF TREATMENT											
				1 Week			3 Weeks			5 Weeks			6 Weeks		
	No. of Men	Positive Slides		No. of Men	Positive Slides		No. of Men	Positive Slides		No. of Men	Positive Slides		No. of Men	Positive Slides	
		No.	%		No.	%		No.	%		No.	%		No.	%
Propionate-propionic acid ointment	39	33	85	35	16	46	30	6	20	30	8	27	38	7	18
Undecylenate-undecylenic acid ointment	40	35	88	37	18	49	36	12	33	36	11	30			
Control—Talcum Powder and ointment	41	38	93				40	19	48				37	15	41

It was unfortunate that the undecylenate-undecylenic acid treatment group could not be observed after 6 weeks of treatment.

In Table V are recorded the results of the cultures made from the 2 treated groups. The small number of positive cultures obtained from groups showing such large percentages of positive slides for fungi was disappointing and made it impossible for us to draw decisive conclusions in regard to the effectiveness of the 2 ointments on the various species of organisms isolated. However, from this data there is no indication that one species is more resistant to treatment than any other species.

The effects of the propionate-propionic acid ointment and the un-

VIDUAL INGREDIENTS.

(Agar cup-plate technique of Ruehle and Brewer.) Clear areas about cups represent zones of inhibition

TABLE V

Comparing the Effects of Propionate-Propionic Acid Ointment and Undecylenate-Undecylenic Acid Ointment on Species of Fungi

TREATMENT GROUP	ONSET							AFTER 1 WEEK							AFTER 3 WEEKS							AFTER 5 WEEKS						
	No. of Men	Positive Cultures						No. of Men	Positive Cultures						No. of Men	Positive Cultures						No. of Men	Positive Cultures					
		T. Menta.		T. rubrum		E. floc.			T. Menta.		T. rubrum		E. floc.			T. Menta.		T. rubrum		E. floc.			T. Menta.		T. rubrum		E. floc.	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
Propionate-propionic acid ointment	39	3	8	8	20	1	3	35	3	8	5	14	1	3	30	0	0	0	0	0	0	30	3	1	3	1	0	0
Undecylenate-undecylenic acid ointment	40	9	22	8	20	1	3	37	3	8	3	8	0	0	36	1	3	3	8	0	0	36	3	8	1	3	0	0

decylenate-undecylenic acid ointment on the clinical manifestations of Tinea pedis are recorded Table VI and Table VII, respectively.

TABLE VI

Demonstrating the Effect of Propionate-Propionic Acid Ointment on the Clinical Manifestations of Tinea Pedis

	TOTAL NO.	INTERTRIGO		MACERATION		SCALING		FISSURING		DENUDATION		DYSHIDROSIS		CLEAR		CLEAR EXCEPT FOR SCALING	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
First Examination	39	19	49	20	51	31	79	16	49	0	0	1	3	0	0		
After 1 Week's Treatment	35	3	9	5	14	25	71	5	14	0	0	0	0	6	17	12	34
After 3 Weeks' Treatment	30	0	0	4	13	21	70	3	10	1	3	1	3	6	20	19	63
After 5 Weeks' Treatment	30	4	13	7	23	13	43	12	40					8	27	10	33
After 6 Weeks' Treatment	38	0	0	2	5	20	53	6	16					16	42	27	71

TABLE VII

Demonstrating the Effect of Undecylenate-Undecylenic Acid Ointment on the Clinical Manifestations of Tinea Pedis

	TOTAL NO.	INTERTRIGO		MACERATION		SCALING		FISSURING		DENUDATION		DYSHIDROSIS		CLEAR		CLEAR EXCEPT FOR SCALING	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
First Examination	40	20	50	20	50	35	88	17	43	0	0	2	5	0	0		
After 1 Week's Treatment	37	3	8	13	35	32	86	8	21	1	3	2	5	3	8	9	24
After 3 Weeks' Treatment	36	6	18	8	22	30	83	9	25			1	3	4	11	12	33
After 5 Weeks' Treatment	36	2	6	7	19	17	47	9	25					14	39	14	39

At the first examination the 2 groups showed a similar distribution of clinical findings. These findings were recorded for each man at the time of the first examination. The same method of tabulating ob-

servations was followed in the examinations made 1, 3, 5 and 6 weeks after onset of treatment. This method of collecting data was advised by Dr. Lowell J Reed.

At the conclusion of the experiment it was impossible to state that one ointment was superior to the other. However, if the clinical manifestations of scaling were removed from the tabulations, the propionate-propionic acid ointment was significantly superior to the undecylenate-undecylenic acid ointment in efficacy. Because scaling is not always a manifestation of fungous infection its omission from the compilation of data is permissible.

When the data were examined after scaling had been deleted, it was noted that after 3 weeks of treatment with the propionate-propionic acid ointment 63 per cent had become "clear", while only 33 per cent had become "clear" in the undecylenate-undecylenic acid treated group. The fact that after 6 weeks of treatment the percentages becoming clear in the propionate-propionic acid group were still rising is of further significance.

It was unfortunate that the undecylenate-undecylenic acid treated group could not have been observed after 6 weeks. The carelessness displayed by the men in following instructions after about 5 weeks of treatment showed up in the results here as well as in the results recorded in Table VI.

DISCUSSION

The development of the fatty acids as fungistatic and fungicidal agents has run an interesting course. First the baking (5) and the dairy industries (6, 7, 8, 9) developed the fatty acids, particularly the sodium and calcium salts of propionic acid, as inhibitors of molds. Then appeared the papers of Peck and Rosenfeld (10), Peck et al (11), Keeney (1), and Keeney and Broyles (2), calling to the attention of the medical profession the possible usefulness of the fatty acids, particularly sodium propionate in the treatment of cutaneous mycotic infections. An recently, we have studied (3) more extensively, by in vitro methods, the fungistatic and fungicidal properties of many fatty acid salts. We concluded from these latter results that sodium caprate and sodium undecylenate should be effective in the treatment of the superficial mycotic infections.

Before our in vitro studies were completed we had begun the clin-

ical investigation of sodium undecylenate. The choice of sodium undecylenate over sodium caprate was stimulated by Dr. J. Gardner Hopkins who had been conducting studies on dermatophytosis at Fort Benning, Georgia. Dr. Hopkins, independent of and prior to us, had arrived at the conclusion that undecylenic acid or a salt of undecylenic acid might prove to be efficacious in the treatment of Tinea pedis.

At the onset we began to study the clinical effectiveness of a 10 per cent sodium propionate ointment prepared in a petrolatum base and 1 per cent sodium undecylenate ointment prepared in a petrolatum base. The Fort Benning group began to study the effectiveness of a 5 per cent triethanolamine undecylenate ointment. The results obtained with the 5% triethanolamine undecylenate ointment appeared superior to those obtained with either the 10 per cent sodium propionate or the 1 per cent sodium undecylenate ointments. Dr. Hopkins had prepared his ointment in a water soluble base ("carbowax") and we both thought that his superior results were due, in part, to better penetration. It was then that we adopted his undecylenate ointment formula and increased the concentration of undecylenic acid from 5 to 10 per cent. It was then, too, that we decided to change our sodium propionate ointment. The concentration of sodium propionate was increased from 10 to 16.4 per cent, propionic acid was added to lower the pH to 5.5 and "carbowax" was employed as a base instead of petrolatum.

With the propionate-propionic acid and the undecylenate-undecylenic acid ointments we began to study their comparative inhibitory effectiveness in vitro against *Trichophyton mentagrophytes*, #640, staphylococcus aureus and beta hemolytic streptococcus. And concomitantly we began our study on the comparative effectiveness of the 2 preparations in the treatment of Tinea pedis.

The propionate-propionic acid ointment was superior to the 5 and 10 per cent undecylenate ointments in its inhibitory effect on *Trichophyton mentagrophytes*. The difference in the zone of inhibition produced by the propionate-propionic acid and 10 per cent undecylenate-undecylenic acid ointments was not great but the difference between the propionate-propionic acid and the 5 per cent undecylenate-undecylenic acid ointments was appreciable.

More surprising still were the results of the in vitro tests with

staphylococcus aureus. Ten per cent ammoniated mercury ointment and 5 per cent sulfathiazole ointment are commonly used locally in the treatment of cutaneous bacterial infections. Both ointments were inferior in vitro to the propionate-propionic acid ointment. On the other hand, the inhibitory effect of both the 5 and 10 per cent undecylenate-undecylenic acid ointments for staphyloccoccus aureus was inferior to the 10 per cent ammoniated mercury and the 5 per cent sulfathiazole ointments. The one-half and full strength Whitfield's ointment were inferior to all other preparations with the exception of 0.5 per cent tyrothricin ointment which failed to exhibit an antibacterial effect.

In regard to beta hemolytic streptococcus the propionate-propionic acid ointment was again superior to all other ointments in its inhibiting effect in vitro. The difference in the zones of inhibition produced by the various ointments was not great, but the results clearly demonstrated that by such in vitro tests 10 per cent ammoniated mercury and 5 per cent sulfathiazole ointments were definitely not superior but inferior in efficacy to the propionate-propionic acid and undecylenate-undecylenic acid ointments. Full strength Whitfield's ointment was fairly effective against beta hemolytic streptococcus. Tyrothricin ointment in a concentration of 0.5 per cent was the least effective.

There is no doubt that sodium propionate and propionic acid and undecylenic acid are the active fungistatic and antibacterial principles in their respective ointments. This was clearly demonstrated by the results presented in Table II and Table III.

With these data from in vitro experiments we have clearly demonstrated that the propionate-propionic acid and the undecylenate-undecylenic acid ointments possess both a fungistatic and an antibacterial effect. The remarkable fact disclosed is that the propionate-propionic acid ointment is not only a superior fungistatic agent, but that it is also superior in its antibacterial effect to preparations that are commonly used in the treatment of cutaneous bacterial infections. It is impossible of course to assume a similar in vivo effectiveness for the propionate-propionic acid ointment but the clinical potentialities offered by this preparation at the moment are extraordinary.

It has been impossible for us to absolutely demonstrate that either

the propionate-propionic acid or the undecylenate-undecylenic acid ointment was superior to the other in the treatment of Tinea pedis. However, by excluding the clinical manifestations of scaling from our data we have shown the propionate-propionic acid ointment superior to the undecylenate-undecylenic acid ointment. This omission of scaling is permissible in that scaling is not always a manifestation of Tinea pedis. But we are hesitant in recommending the propionate-propionic acid ointment before the undecylenate-undecylenic acid ointment on these grounds alone and it is not until the in vitro fungistatic and antibacterial effects are considered that it is possible to favor the propionate-propionic acid ointment.

Thus far we have not encountered irritation or sensitization from the daily and protracted use of either preparation. A temporary sensation of stinging or burning results from the application of either preparation on an area of skin where the epithelium has been denuded, but this has not been a momentous complaint.

Finally we do not wish to infer that with the use of either propionate-propionic acid or undecylenate-undecylenic acid ointment other methods of treating Tinea pedis be dismissed. The use of keratolytic agents, the maintenance of good foot hygiene and the procedure of opening widely vesicular lesions on the soles of the feet must, as the occasion demands, supplement the use of either the propionate-propionic acid or the undecylenate-undecylenic acid ointment.

SUMMARY

We have studied the clinical effectiveness of propionate-propionic acid ointment and of undecylenate-undecylenic acid ointment in the treatment of Tinea pedis. We have compared the in vitro fungistatic and antibacterial effects of the propionate-propionic acid ointment and 5 and 10 per cent undecylenate-undecylenic acid ointments with one-half and full strength Whitfield's, 10 per cent ammoniated mercury, 5 per cent sulfathiazole and 0.5 per cent tyrothricin ointments.

The propionate-propionic acid ointment in vitro was superior in its fungistatic effect on *Trichophyton mentagrophytes*, to the 5 and 10 per cent undecylenate-undecylenic acid, one-half and full strength Whitfield's, 10 per cent ammoniated mercury, 5 per cent sulfathiazole and 0.5 per cent tyrothricin ointments.

The propionate-propionic acid ointment in vitro was superior in its antibacterial effect on staphylococcus aureus (coagulase positive) and beta hemolytic streptococcus (Group A), to 10 per cent ammoniated mercury, 5 per cent sulfathiazole, 5 and 10 per cent undecylenate-undecylenic acid, one-half and full strength Whitfield's and 0.5 per cent tyrothricin ointments.

The sodium propionate and propionic acid and the undecylenic acid are the active fungistatic and antibacterial principles in their respective ointments.

Propionate-propionic acid and 10 per cent undecylenate-undecylenic acid ointments prepared in a "carbowax" base gave comparable results in the treatment of Tinea pedis. When the clinical manifestations of scaling were removed from the data, the propionate-propionic acid ointment was statistically superior to the undecylenate-undecylenic acid ointment. Neither preparation produced irritation nor sensitization.

Both the propionate-propionic acid and the undecylenate-undecylenic acid ointments satisfy the qualifications, enumerated in this report, of an ideal preparation for the treatment of Tinea pedis; that is, (1) fungistatic or fungicidal activity, (2) antibacterial effect, (3) penetration, and (4) lack of irritation and sensitization.

We recommend the use of either propionate-propionic acid or undecylenate-undecylenic acid ointment in the treatment of Tinea pedis and we feel that either preparation is an improvement over any one method of treatment in use at the present time. Because of the superior in vitro fungistatic and antibacterial effect and the slight superior clinical effect of the propionate-propionic acid ointment, we are inclined to recommend this preparation in preference to the undecylenate-undecylenic acid ointment.

We wish to stress that the use of keratolytic agents, the maintenance of good foot hygiene, and the procedure of opening widely vesicular lesions on the soles of the feet are measures which must not be lost sight of and which should supplement the use of either the propionate-propionic acid or the undecylenate-undecylenic acid ointment.

Subsequent to submitting this paper for publication we have demonstrated by in vitro experiments that the propionate-propionic acid ointment was as effective as penicillin ointment against beta hemolytic streptococcus (group A) but

less effective against staphylococcus aureus (coagulase positive). The penicillin ointment was prepared in a carbowax base so that each gram of ointment contained 500 units of penicillin.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

The Pathogenesis of Tuberculosis. By ARNOLD R. RICH. 900 pp. $10.50. Charles C. Thomas, Springfield & Baltimore, 1944.

Medical books conveniently may be divided into four classes. The first class comprises digests, summaries, compendiums, guides and manuals. The avowed purpose of these books is to furnish brief information about the fundamental facts of the chosen subject. They are useful tools for hasty reference or superficial review. Their preparation requires chiefly diligence although they are improved when diligence is supplemented by experience in the field and by skill in presentation. Now and again an author of wide experience with an intelligent grasp of the subject matter will produce a volume of merit which may be reissued in successive editions and for many years sustain a well deserved reputation.

The second class comprises the text-books. These are written by teachers, usually by those who have attained a degree of eminence in their particular branch. They are designed to give an orderly and adequate, though not detailed, survey of the subject. Their usefulness depends upon the intelligence and skill with which knowledge and experience are assembled and presented. A renowned teacher may succeed in giving a text-book unusual distinction by impressing upon it the stamp of his great personality. For the most part they are ephemeral and one is rapidly succeeded by another.

The third class consists of systems and handbooks. These are more pretentious than the text-book and are supposed to give authoritative and comprehensive information, to discuss matters not yet settled and divergent points of view, and furnish reference to original sources. Under the direction of an editor each department of the subject is covered by a specialist in the field. Since there are many contributors, the articles vary in value with the competency of the respective authors. These volumes are books of reference found usually in libraries where they are consulted chiefly by those with special interest in the particular subject.

In the fourth class are the monographs, books which present and discuss in detail the existing knowledge of a special subject or of some particular aspect of a special subject. Usually a single disease is selected or a group of closely related diseases. Sometimes a type of reaction may be considered which is observed under many different circumstances or the pathological physiology of a symptom that occurs in many diseases. Monographs have a distinctive flavor and a special place among medical books. Many of them do not rise above the commonplace but the best of them are the jewels of medical literature. Not only do they give fuller information about the chosen subject than can be found elsewhere but many are infused with the enthusiasm and adventurous spirit of the author.

The book of Dr. Rich on the Pathogenesis of Tuberculosis belongs in this

fourth class and among the very best of the class. The title is well chosen for it discloses at once the purpose of the book and at the same time arouses interest. The desire of every pathologist is to explore pathogeneses, and to unravel the cause, and manner of development and progression of disease, is the flower of his labor. It required courage to undertake so formidable a task as the exposition of the present state of our knowledge about the pathogenesis of tuberculosis. There is hardly another disease which has been so diligently investigated, about which so much has been written, and which presents so many problems still unsolved. Although the book is written in a clear and facile style that leaves no impression of labor, it must have cost the author toil and sweat to familiarize himself with the vast literature on tuberculosis. Over 1400 references are cited in the text all of which, in addition to many others, the author, as he explains in the preface, personally examined at first hand. This is a comforting reassurance since sometimes extensive bibliographies, designed to give the appearance of prodigious industry or uncommon scholarship, are references merely copied from the Index Medicus without having been read. However, the number of references quoted, even though all have been read, is of itself no index to the value of a book. The vital point is the use the author makes of this erudite information. A perusal of Dr. Rich's book convinces us that with him its use is not a scholarly affectation or embellishment. Each reference is appropriately cited in the text at the point where the article bears pertinently upon the matter under discussion.

It would do a great injustice to this remarkable book were the reviewer to create the impression that it is merely a compilation of our present knowledge of the pathogenesis of tuberculosis. That it assuredly is, but it is something much more than that. The spirit of the book, its plan and form, give it distinction. A thorough knowledge of the subject, apparent on every page, is the substance which is moulded into a clear and engaging account of all the details of tuberculous infection and disease. The style is clear and fluent, so that it is a pleasure to read the book and each exposition and argument can be clearly understood without the annoyance of pausing to decipher clumsy or ambiguous sentences, or to unravel and rearrange poorly constructed arguments. Loop holes in our knowledge are exposed and points about which opinions differ are fully discussed. The author does not hesitate to state his own opinion but always gives the reason for his preference and treats the opinions of others with respect.

The reviewer has got great satisfaction and pleasure from this book and is confident it will be equally useful and pleasing to others. He considers it to be one of the outstanding medical publications of recent years.

L. H.

Experimental Basis for Neurotic Behavior. By W. H. GANTT. 198 pp. Illus. $4.50. George Banta Publishing Company, Wisconsin, 1944.

The author's studies of a 12 year period in the Pavlovian Laboratory of the Phipps Psychiatric Clinic on the nervous disturbances of dogs are presented in this excellent monograph. One dog (Nick) has been observed for 12 years with

more than 15,000 conditioned reflexes separately recorded. The piecing together of this enormous amount of data results in a most valuable contribution to our understanding of chronic disturbances in behavior. The comparison of the results on Nick with those on two other dogs, who were observed for shorter periods, shows the individual variations in artificially produced neuroses in dogs. The introductory chapters give the historical development of Pavlov's concepts and a general description of the methods. The step by step loss of nervous balance is described in the next chapters. The life history of three dogs subjected to the same difficult routine is presented in great detail. This is followed by categorical enumeration and interpretation of the symptoms according to physiological systems. The results of therapy and the existence of functional types are discussed in the next chapters. The concluding chapter is a most interesting attempt to elucidate the psychopathological mechanisms operative in the nervous breakdown of the experimental animals. The author has submitted his data to three psychiatrists representing different schools and has asked them to analyze these data in the light of their special conceptual background. The psychiatrists are Dr. N. Ischlondsky, representing the reflexologists, Dr. L. Saul, representing the psychoanalysts and Dr. A. Leighton, a pupil of Adolf Meyer and a distinguished anthropologist. The answers of these experts are quoted verbatim and are supplemented by remarks of the author. In this way the reader is made familiar first with the experimental material and the author's own interpretations and is then provided with explanations in the light of the three great schools of psychopathology; the Pavlovian, the Freudian and the Meyerian. A comprehensive bibliography and an index conclude the book, the format of which is very pleasing.

Pavlov's method of conditioned reflexes is one of the most powerful tools ever developed for the study of the functions of the central nervous system. By availing themselves of this tool in a masterly fashion the author and his collaborators have obtained important results and have advanced our knowledge of the physiological basis of neurotic behavior considerably. The book is warmly recommended, not only to students of psychobiology and psychiatry but to everybody interested in the advance of physiological methods and concepts.

W. F.

Safe Convoy. By WILLIAM J. CARRINGTON. 239 pp. $2.50. J. B. Lippincott Company, Philadelphia & New York, 1944.

Dr. Carrington's recently published book adds not only a new, but somewhat different chapter to the volume of available maternal guides.

The entertaining and pertinent puncturing of innumerable obstetrical superstitions together with the many historical references found throughout the book make excellent constructive information.

In "Safe Convoy" the basic principles of maternal care and hygiene are at times submerged in an unusually detailed sea of pathological obstetrics. This will appeal to that ever increasing group of mothers who wish to consider themselves medical experts: the conservative numbers of expectant mothers may well find, in the reading unnecessary worry and sleepless anticipation.

The chapters on the newborn infant and its care are excellent, and in much more detail than usually found in Mothers' Handbooks.

W. D. E.

Practical Malaria Control. By CARL GUNTHER. 91 pp. $2.50. Philosophical Library, Inc., New York, 1944.

Nothing is more stimulating to the student of malaria control than to review the experiences of those who have battled this ubiquitous disease in newly developed areas. Gunther's book accordingly furnishes an interesting bit of reading. As Gunther himself says in his foreword, "It does not pretend to be a treatise on malaria, but is merely an outline of the practical problems involved." It does effectively outline the problems colored by the author's experience in New Guinea, but the reviewer was left with the impression that these problems were more readily solved on the pages of the book than is actually the case in the field. The suggestion that a mosquito food barrier in terms of animals and immune (native) populations be placed between the breeding places and the non-immune (i.e. caucasians or other recent immigrant) populations is a fascinating idea and well presented but the complications of this procedure and differences in the habits of the vectors in various parts of the world are such that it would be surprising if much were accomplished by the procedure exceept in unusual circumstances. This slim volume is well worth reading even though, as the author says, "some (of the opinions) are radical, some unscientific, and most are expressed dogmatically."

G. F. O.

Language and Thought in Schizophrenia. By J .S. KASANIN. 133 pp. $2.00. University of California Press, Berkeley and Los Angeles, 1944.

This little book of collected papers by several authors does much to clarify, for the medical man and the psychiatrist in particular, the schizophrenic reaction and the understanding of psychiatric patients generally through highly specialized, quite technical, but thoroughly comprehensible discussion of several interesting aspects of schizophrenic language and thought.

N. L. B.

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Diseases of the Digestive System. Edited by SIDNEY A. PORTIS. 909 pp. Illus. $11.00. Lea & Febiger, Philadelphia, 1944.

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